JOURNAL OF DAIRY SCIENCE

Contents

Studies of the Growth and Blood Composition of Dairy Calves Fed Remade Skim Milk after Three Days of Age. L. R. ARRINGTON AND P. M. REAVES 1

The Effect of Penicillin upon the Livability, Glycolysis, and Bacterial Content of Bovine Semen. J. O. ALMQUIST, W. T. S. THORP, AND C. B. KNODT 11

The Effect of Conditions of Storage on the Viscosity of Sweetened Condensed Milk. B. H. WEBB AND C. F. HUFNAGEL 21

Determination of Cheese Lipase. MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE 31

Lipase Activity during Making and Ripening of Cheddar Cheese. MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE 39

Determination of Cheese Proteinase. MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE 47

Proteinase Content of Cheddar Cheese during Making and Ripening. MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE 55

The Heritability of Official Type Ratings and the Correlation between Type Ratings and Butterfat Production of Ayrshire Cows. W. J. TYLER AND GEORGE HYATT, JR. 63

Variations in Type Ratings of Individual Ayrshire Cows. GEORGE HYATT, JR., AND W. J. TYLER 71

The Utilization of Lactose by the Dairy Calf Fed Normal or Modified Milk Diets. JORGE ROJAS, B. S. SCHWEIGERT, AND I. W. RUPEL 81

Association Announcement 89

Abstracts of Literature A1

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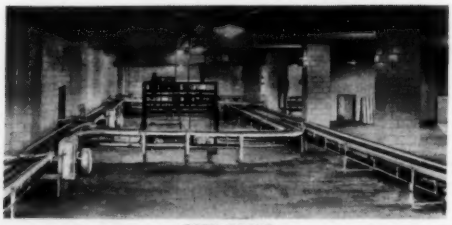

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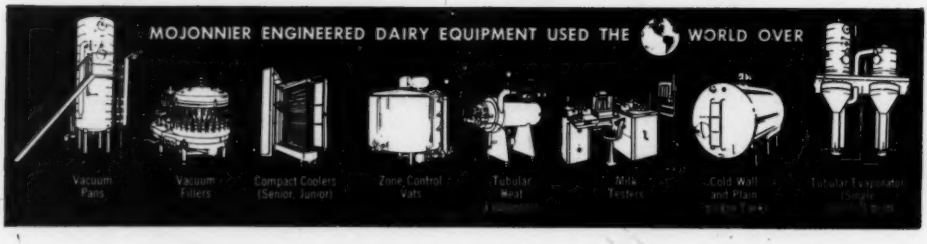

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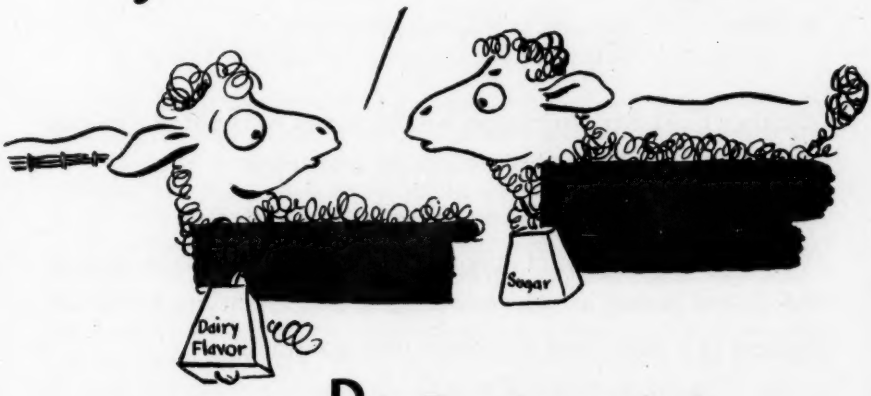

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Another Field Representative will be added to the staff, making a total of seven National Field Representatives.

A survey is to be made of milk markets and producers throughout the United States, in order to obtain data necessary to the development of a sound and aggressive milk program.

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXI JANUARY, 1948 NUMBER 1

STUDIES OF THE GROWTH AND BLOOD COMPOSITION OF DAIRY CALVES FED REMADE SKIM MILK AFTER THREE DAYS OF AGE[1]

L. R. ARRINGTON[2] AND P. M. REAVES

Dairy Husbandry Department, Virginia Polytechnic Institute, Blacksburg

The common method of feeding dairy calves for the first few weeks consists of feeding whole milk or partially skimmed milk for a limited time and then changing to skim milk. Other methods reported (3, 4, 10) involve changing abruptly to skim milk after the colostrum period and have proved satisfactory when the skim milk has been supplemented with vitamins A and D. Less success has been reported, however, with feeding remade skim milk. Morrow (11), in comparing fresh skim milk with remade skim milk, found that calves fed remade skim milk did not make as good growth as those fed fresh skim milk. Digestive disturbances also were noted in those calves fed the remade skim milk. Knott *et al.* (6) fed remade skim milk after 2 to 6 weeks of age and thereafter fed skim milk powder in the grain ration; they secured normal growth.

The partial success with feeding remade skim milk indicated the need for additional study of this method of feeding. The effects of this method would be expected to be exhibited, first, in the growth of the animals. The low-fat intake of the ration indicated a study of the blood fat content. Allen (2) has reported that the blood fat of dairy cattle is closely associated with feeding conditions. He reported that the blood of the newborn calf contains only a trace of fat, but the fat increases rapidly with the ingestion of food. This method of feeding also suggested a study of the blood carotene and vitamin A, since the skim milk must be supplemented with carotene or vitamin A.

EXPERIMENTAL PROCEDURE

Seven Holstein and six Jersey male calves from the Virginia Polytechnic Institute dairy herd were used for this experiment. The seven Holstein calves and five of the Jersey calves were removed from their dams on the

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[1] Data taken from thesis presented by L. R. Arrington as partial requirement for degree of Master of Science in Dairy Husbandry, 1941.

[2] Now at the University of Florida, College of Agriculture.

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third day after birth and placed on the experimental ration. One of the Jersey calves was 10 days old when placed on the experiment and had received whole milk until that time. Each calf was housed in an individual stall throughout the experiment. An additional Holstein calf was used as a control, being handled in the same manner as the others except that fresh whole milk was fed in place of remade skim milk.

The experimental ration of remade skim milk was prepared by mixing 1 lb. of skim milk powder (spray process) with 9 lb. of warm water. This was fed at the rate of 1 lb. of milk daily per 10 lb. of body weight. A grain mixture was supplied as soon as the calves would eat it. The mixture consisted of 200 lb. of corn, 100 lb. of wheat, 250 lb. of whole oats, 250 lb. of wheat bran, 100 lb. of distillers grain, 100 lb. of linseed meal, 10 lb. of steamed bone meal, and 10 lb. of salt. Chopped red clover and alfalfa hay was supplied *ad libitum* as early as the calves would eat it.

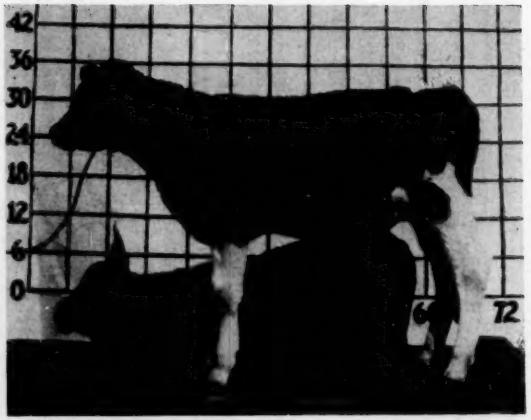
One group of calves was fed a carotene concentrate;[3] the other group was fed cod-liver oil as the supplement. Those calves receiving the carotene supplement were given 20 drops of viosterol daily as a protection against vitamin D deficiency. The rate of carotene feeding was adjusted weekly with the increase in body weight. These concentrates were fed by mixing with the milk at the evening feeding.

Body weight and height at withers were taken at 3 days of age, when each calf was placed on the experiment, and each week thereafter, and blood fat determinations were made using the method described by Allen (1). Blood carotene determinations also were made weekly by the method of Moore (7), and blood vitamin A determinations were made weekly on six of the calves by the method of Kimble (5).

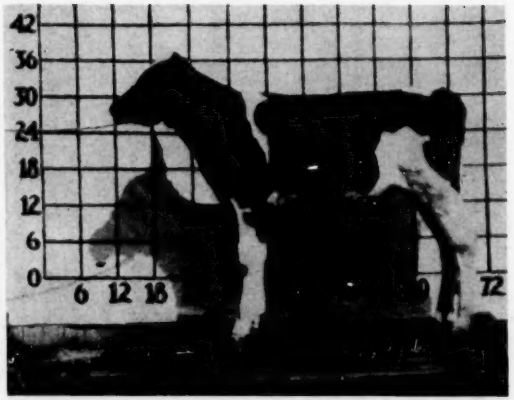
RESULTS

Six of the seven Holstein calves lived through the experimental period. Only one of the Jerseys (no. 812) lived through the experimental period, but this one was 10 days old when placed on experiment and had received whole milk until that time. Another Jersey calf (no. 813) lived until 14 weeks of age and died of bloating. Gain in body weight for this calf was slightly below normal. Since four of the six Jersey calves died within 2 to 4 weeks following the change to remade skim milk, they evidently could not withstand the abrupt change to the experimental ration or did not have the reserve to carry them over until they consumed feed other than milk. In each case where the calf died, scours occurred, and there was a steady loss in weight. Scours occurred in some of the other calves also, and it became necessary to reduce the milk intake. After the first 2 to 3 weeks, scours was much less prevalent, and animals appeared to be in better general health. There were no evidences of vitamin A or D deficiency.

[3] A commercial carotene preparation, Carotene—Type P–25, prepared and supplied by General Biochemicals, Inc.



Calf no. 819—Holstein fed carotene and viosterol. Age, 16 weeks; weight, 248 lb.



Calf no. 821—Holstein fed cod-liver oil. Age, 16 weeks; weight, 240 lb.

FIG. 1. Typical calves from each group receiving a supplement.

Table 1 shows the weekly weight of each calf throughout the experimental period. The Holsteins showed little or no gain for 2 or 3 weeks following the change to the experimental ration, and the Jerseys lost weight during this time. The retarded rate of gain is attributed to the low T.D.N. intake of the ration, since increases were nearly normal soon after grain and hay were consumed regularly. The intake of nutrients was below the requirement according to the Morrison Standard (9) until grain and hay were taken.

With the normal intake of grain and hay at about 6 weeks of age, increases in body weight were almost normal, and at 16 weeks of age the calves were practically normal size according to figures of Ragsdale (12).

Gains in height at withers were slightly below the normal rate (12) for the first few weeks. After about 3 weeks the Holsteins showed a normal increase in height, and after about 5 weeks the increase for the Jerseys compared favorably with the normal. There was no apparent difference in body weight and height at withers between the group fed carotene and the group fed cod-liver oil. Calves no. 819 and 821 were selected as typical calves from each group. Figure 1 shows a photograph of each.

Table 2 shows the weekly blood fat content of each calf. There were variations from week to week and there were differences among the animals, but there was no significant difference between the group fed carotene and the group fed cod-liver oil. Blood fat of the experimental calves was lower than that of the control calf fed whole milk. With the increase in intake of grain and hay, blood fat increased.

Blood carotene levels in those calves fed the carotene supplement were well above those reported as minimum requirements (8). The amount of blood carotene was considerably higher in this group than in the group fed cod-liver oil (table 3). There were slight variations from week to week in each group, but a general trend to a higher level toward the end of the experiment, when the calves were eating more hay, was apparent.

Blood vitamin A determinations were made on only six of the experimental animals, including the control. The plasma concentrations of those calves fed cod-liver oil as a supplement were higher than those of either the carotene group or the control calf (table 4). Concentrations for the control calf ranged intermediate between those of the cod-liver oil group and the carotene group.

SUMMARY

Seven Holstein and six Jersey male calves were fed an experimental ration of remade skim milk supplemented with vitamin A and D in the form of carotene and viosterol or cod-liver oil. The seven Holstein calves and five of the Jersey calves were placed on the experimental ration at 3 days of age. One of the Jersey calves received whole milk until 10 days of age. Grain and hay were fed as soon as the calves would eat it.

TABLE 1

Body weight of experimental calves

No. of calf	Breed	3rd day	Age (weeks)															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
										(*lb.*)								
817*	H	75	75	77	84		90	103	113	125	140	145	155	179	193	207	219	237
								Carotene group										
810	H	100	93	97	91	Died												
812	J		56	54	55	55	62	70	76	85	90	103	109	119	132		145	167
814	J	45	47	45	Died													
815	H	92	93	99	108	112	124	125	135	149		168	183	197	211	224		
819	H	102	102	104	108	119	127	132	143	150	163	171	182	198	206	210	235	248
820	H	97	97	98	103	108	112	120	127	136	147	157	164	177	186	192	209	228
822	H	90		93	100	102	106	113	123	125	143	152	Experiment terminated					
823	J	47	45	44	43	41	Died											
824	J	51	50	47	41	Died												
								Cod-liver oil group										
813	J	55	52	50	51	55	62	64	74	82	94	96	105	115		128	Died of bloat	
816	J	50	44	43	Died													
818	H	84	82		80	85	91	99	113	120	133	142	153	164	179	185	194	
821	H	102	103	104	106	112	120	126	137	145	154	165	180	190	199	200	227	240

*Control calf.

TABLE 2

Fat content of blood plasma

No. of calf	Breed	3rd day	Age (weeks) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
									(*mg./ml.*)									
817*	H	29	49	57	78		87	140	135			123	154	149	164		146	142
									Carotene group									
812	J								76	39	32	47	48	86	87	118	69	
815	H				15	16	14	31	35	55	46	83	70	52	29	50		
819	H		22	42		24	24	23	40	35	83	65	128	144	178	218	208	155
820	H	40	27		11	11	23	38	24	44	44	93	99	158	189	190	132	133
822	H		24	25	34	65	69	105	106	89	49	64	Experiment terminated					
823	J	47	110	78			Died											
824	J	20	63			Died												
									Cod-liver oil group									
813	J							53	27	20	38	22	53	39			Died (of bloat)	
816	J	24	20	10	Died													
818	H	25	19	20	22	16	29		24	31	31	36	46	51	75	129	131	
821	H	50	26		32	23	12	15	26	27	54	110	87	157	180	173	126	119

*Control calf.

TABLE 3

Carotene content of blood plasma

No. of calf	Breed	3rd day	Age (weeks)															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
									(μg./ml.)									
817*	H	0.26	0.16	0.15	0.08		0.11	0.21	0.08		0.16	0.22	0.54	0.48	0.49	0.45	0.59	0.62
									Carotene group									
812	J						0.15	0.39	0.39	0.23	0.54	0.40	0.41	0.74	0.58	0.42	0.85	0.59
815	H	0.34	0.10	0.20	0.11	0.24	0.25	0.25	0.45	0.26	0.11	0.48	0.45	0.31		0.58		
819	H		0.12	0.18		0.42	0.45	0.49	0.44	0.44	0.42	0.66	0.92	1.34	1.03	1.03	1.12	0.76
820	H	0.12	0.11		0.30	0.18		0.38	0.35	0.35	0.50	0.58	0.84	0.72	0.70	0.80	0.49	0.47
822	H		0.25	0.17	0.27	0.26	0.41	0.42	0.46	0.45	0.15	0.16	Experiment terminated					
									Cod-liver oil group									
813	J				0.10	0.20	0.25	0.15	0.30	0.28	0.14	0.34		0.21	0.21	Died (of bloat)		
818	H	0.05	0	0	0.09	0.03		0.04	0.05		0.15	0.16	0.16	0.30	0.47	0.61		
821	H	0.23	0.07		0.03		0.03	0.04	0.23	0.12	0.16	0.14	0.14	0.11	0.12	0.08	0.14	

* Control calf.

TABLE 4

Blood vitamin A

No. of calf	Breed	Age (weeks)															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
										(μg./ml.)							
817*	H									0.08	0.10		0.07	0.13	0.18	0.13	0.10
								Carotene group									
819	H			0.08	0.05		0.04	0.06	0.08	0.05			0.04	0.07	0.06	0.06	0.08
820	H			0.09	0.07		0.05	0.07	0.07	0.07	0.06	0.04	0.03	0.05	0.05	0.10	0.09
822	H	0.15	0.06	0.03		0.03	0.03	0.04	0.02	0.03	Experiment terminated						
								Cod-liver oil group									
818	H							0.23	0.21		0.14	0.21	0.23	0.18	0.13	0.15	
821	H			0.16	0.10		0.17	0.17	0.12	0.11	0.10	0.11	0.16	0.17	0.15	0.19	0.18

* Control calf.

Six of the seven Holstein calves lived through the experimental period of about 16 weeks. Growth rates were below normal for the first few weeks, but Holstein calves were practically normal size at 16 weeks of age. Four of the six Jersey calves died within 2 to 4 weeks following the change to the experimental ration. One of the other Jerseys died at 14 weeks of age from bloat, and only one Jersey lived through the 16-week experimental period. This one received whole milk until 10 days of age. There was no difference in growth rates between the groups fed the different vitamin supplements.

Blood fat content was lower in calves fed the experimental ration than that of the control calf fed whole milk. As the quantity of grain and hay consumed increased, the blood fat increased.

Blood carotene was higher in calves receiving the carotene supplement than that of the control calf on whole milk or that of the experimental calves receiving cod-liver oil as the supplement.

Blood vitamin A of calves fed cod-liver oil was higher than that of the control calf or that of the group receiving carotene as the supplement.

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THE EFFECT OF PENICILLIN UPON THE LIVABILITY, GLYCOLYSIS, AND BACTERIAL CONTENT OF BOVINE SEMEN[1,2]

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One of the most important problems in the artificial breeding of dairy cattle at the present time is the control of the bacterial flora of diluted bull semen. While present-day techniques in the collection of bull semen under routine artificial breeding conditions tend to reduce bacterial contamination, it is still impossible to obtain semen which is absolutely free of bacteria. Unless adequate precautions are taken in the preparation and handling of the diluted semen, further contamination may occur. In addition, both undiluted and diluted semen serve as excellent media for bacterial growth.

It is believed that the semen of the bull may serve as a means of transmitting certain genital infections which are related to fertility problems in a dairy herd. The widespread use of artificial breeding has greatly magnified the seriousness of this problem.

On the basis of the foregoing facts, the addition to semen of an antibacterial substance which does not exert an injurious effect on the spermatozoa would be of value in artificial breeding under field conditions.

As early as 1917, Ivanov (3) recognized and investigated the possibility of controlling the spread of infection by adding certain chemical substances, namely, ethyl alcohol, atoxyl, and salvarsan, to contaminated semen. In 1940 Shettles (8) reported that the survival and activity of human spermatozoa were not reduced by sulfanilamide or sulfapyridine in concentrations as high as 160 mg. per 100 ml. of the Baker's fluid used for diluting purposes. However, Knodt and Salisbury (4) were the first investigators to study the feasibility of using certain bacteriostatic compounds to control bacterial growth in bull semen. Using sulfanilamide at levels ranging from 0 to 1,000 mg. per 100 ml. of yolk-citrate diluter, they found that levels of 200 mg. and over controlled the growth of bacteria in stored diluted semen (20 days' storage at 5° C.). They concluded that 300 mg. of sulfa-

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[2] The data contained in this paper are part of a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

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nilamide per 100 ml. of diluter was the most favorable level since it not only prevented bacterial growth but also brought about a significant increase in spermatozoan livability. Further studies by these workers (6) more recently have shown that the addition of sulfanilamide to diluter at this optimal level gave an increase in the fertility of bull semen used in the routine artificial breeding of dairy cows. They attributed the beneficial effects of sulfanilamide on fertility to changes in the metabolism of the spermatozoa rather than to the control of bacterial growth alone.

Recently the possibility of using other antibacterial agents to prevent bacterial growth in bull semen has been reported. In attempts to produce a synthetic diluting medium which would be superior to the egg yolk-buffer diluters commonly used at the present time, Phillips and Spitzer (5) noticed considerable growth of bacteria in some of the preparations. After studying the effect of various antibacterial substances upon the motility of spermatozoa during storage, they recommended that 0.03 per cent of sulfathalidine, sulfasuxidine, or streptomycin be added to their newly developed lipid-glucose-buffer-gum (LGB) diluter to control bacterial contamination. They also mentioned that penicillin was not deleterious to spermatozoan motility but did not recommend its use in their LGB diluter.

Since penicillin is recognized as one of the most effective antibiotic agents in treating a variety of infections and is unique because of low tissue toxicity, it seemed desirable to study its use in bovine semen. Thus laboratory and field experiments were undertaken to determine the effects of penicillin upon the bacterial content of diluted bovine semen and its influence on the livability, metabolism, and fertility of the spermatozoa. The effect of penicillin upon fertility still is under investigation and will be reported later.

EXPERIMENTAL

Effect of penicillin upon spermatozoan livability. Preliminary studies to determine the effect of penicillin upon spermatozoan livability indicated that levels of 2,500 up to 10,000 Oxford units of penicillin per ml. of diluted semen were definitely detrimental. Thus, penicillin was added to 12 samples of bull semen at the rate of 0, 250, 500, 750, 1,000, 1,250, 1,500, and 2,000 Oxford units per ml. of diluted semen. The 12 ejaculates were diluted at the constant rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. This dilution was selected because it represented the average dilution rate being used by the artificial breeding cooperatives in Pennsylvania at the time the experiment was designed. The yolk-citrate diluter was composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g. of $Na_3C_6H_5O_7 \cdot 2H_2O$ in 100 ml. of water distilled over glass. When the design of the experiment involved the addition of penicillin, the desired amount of this substance was dissolved in the sodium citrate solution and mixed with the egg yolk in order to ensure the preparation of a diluter which would have a 1 : 1 ratio of yolk to buffer.

To test the effect of penicillin upon spermatozoan livability, the diluted semen was stored at 4.5°C. and the percentages of actively motile spermatozoa were determined every 2 days for a period of 20 days. At 0, 8, and 16 days of storage, subsamples were taken for glucose and lactic acid determinations, as well as for bacterial counts and penicillin assays.

The 12 ejaculates studied had a mean concentration of 1,147,000 spermatozoa per cubic millimeter, a mean motility of 70 per cent active spermatozoa, and a mean methylene blue reduction time of 7.2 minutes (range 5.0 to 10.0 minutes).

The results of the motility observations are shown in table 1. Each figure represents a mean of 12 ejaculates. Analysis of variance involving a total of 960 motility estimations showed no significant differences in livability between the 0, 250, 500, and 750 unit levels of penicillin. The 1,000,

TABLE 1

The effect of penicillin upon the livability of spermatozoa

Penicillin units per ml. diluted semen	Per cent motile spermatozoa (12 ejaculates)					
	Before storage	After storage at 4.5° C. for				
		4 days	8 days	12 days	16 days	20 days
Control	70	52	41	31	17	11
250	70	52	40	30	16	11
500	70	52	40	27	17	7
750	70	54	40	27	16	7
1000	70	52	39	26	16	6
1250	70	51	37	25	14	4
1500	70	47	33	22	12	4
2000	70	45	32	21	10	2

1,250, 1,500, and 2,000 unit levels of penicillin brought about a highly significant ($P = <0.01$) decline in the ability of the spermatozoa to maintain motility as compared to the untreated controls. Highly significant differences between ejaculates and between storage intervals were found, as well as highly significant ejaculate × treatment and storage interval × ejaculate interactions. Apparently the various ejaculates responded differently to the several levels of penicillin in their livability during storage.

An examination of the mean motility observations for each treatment indicated that there was a more or less uniform decline in livability with increased amounts of penicillin. Thus, the relationship between spermatozoan livability and level of penicillin also was studied by means of regression. Both highly significant linear and curvilinear regressions were obtained (fig. 1). However, a test of significance of departure from linearity showed that the linear regression represented the livability data more accurately. Based on linear regression, the mean percentage of motile spermatozoa during 20 days' storage decreased by 0.9 per cent for each additional 250 units of penicillin as compared to the untreated controls.

Since in routine artificial breeding practically all semen is used by the time it is 6 days of age, an analysis of variance was made which included only the motility data for 2, 4, and 6 days' storage. According to the least differences required for significance, levels of penicillin up to and including 1,000 units did not cause a mathematically significant decrease in livability as compared to the untreated controls, but the levels of 1,250, 1,500, and 2,000 units brought about a highly significant decline in spermatozoan activity.

Effect of penicillin upon the glycolysis of spermatozoa. The studies of Knodt and Salisbury (4) indicated that the percentage recovery of glucose

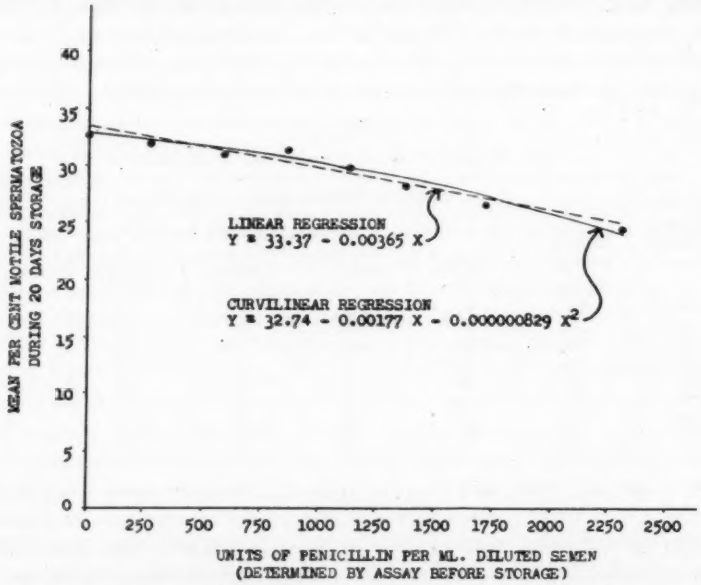

FIG. 1. Relationship of per cent motile spermatozoa during 20 days' storage to level of penicillin, as shown by regression.

as lactic acid was relatively small in diluted bull semen containing no sulfanilamide, but increased when the sulfanilamide was added. In the present study, glucose and lactic acid determinations were made on nine samples of diluted semen stored for 0, 8, and 16 days. Glucose analyses were made according to the method of Somogyi (9), while lactic acid was measured by the method of Barker and Summerson (2). The data are shown in table 2. Analysis of variance involving 216 glucose determinations demonstrated that penicillin significantly reduced the utilization of glucose at all levels. On the other hand, an analysis of the corresponding lactic acid determinations showed no statistically significant differences between the controls and

the various levels of penicillin. The addition of penicillin increased the percentage of glucose loss recovered as lactic acid as compared to the tubes without penicillin. Thus, bacteria may have utilized part of the glucose not recovered as lactic acid in the diluted semen which was not treated with penicillin.

Effect of penicillin upon the bacterial content of diluted semen. Bacterial plate counts were made on five samples of diluted semen using veal infusion agar containing 2 per cent sterile defibrinated ox blood. The samples were plated at dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 with incubation at 37° C. for 72 to 96 hours. Organisms of the coliform group were determined by counts on desoxycholate agar plates which were examined after 24 hours at 37° C. and, if no colonies were present, after an additional 24 to 48 hours. Bacterial counts were made not only on diluted semen after 0, 8, and 16 days of storage, but also on samples of undiluted semen and yolk-citrate diluter stored for the same intervals.

TABLE 2

The effect of penicillin upon the glycolysis of diluted bull semen stored for 16 days at 4.5° C. (mean of 9 determinations)

	Units of penicillin per ml. of diluted semen							
	0	250	500	750	1000	1250	1500	2000
Mg. % glucose* loss	63	46	58	51	43	52	49	44
Mg. % lactic acid gain	46	47	47	49	48	49	46	42
% glucose utilized recovered as lactic acid	73	102	81	96	112	94	94	95

* Reducing substances expressed as glucose.

In the five semen samples studied, no growth of typical coliform colonies on the desoxycholate agar plates was noted.

The data presented in table 3 indicate that penicillin retarded bacterial growth in freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was observed in the tubes without penicillin. The majority of the plates made from both penicillin-treated and untreated semen stored for 16 days possessed a heavy growth of minute colonies which made counting impossible. In four out of 20 possible cases, countable plates were obtained at the 250 and 500 unit levels from the tubes stored for 0 and 8 days. Possibly the raw semen contained bacteria which were relatively resistant to penicillin at these lower concentrations but which were inhibited by the higher concentrations.

The three irregular plate counts obtained in sample numbers 13 and 14 at levels of 1,000, 1,500, and 2,000 units (after storage for 8 days), as well as the heavy growth of pin-point colonies noted at 16 days (table 3), may

TABLE 3

The effect of penicillin upon bacterial growth in diluted semen stored for 8 and 16 days at 4.5° C. (5 ejaculates)

Bull	Sample no.	Bacteria per ml. (in thousands)									
		Undiluted semen	Yolk-citrate diluter	Units of penicillin per ml. of diluted semen							
				0	250	500	750	1000	1250	1500	2000
		Before storage									
B	11	0.2	*	*	*	*	*	*	*	*	*
C	12	7.7	*	0.6	*	*	*	*	*	*	*
B	13	1.6	*	0.7	*	*	*	*	*	*	*
A	14	960	*	76	41	*	*	*	*	*	*
B	15	850	*	50	54	25	*	*	*	*	*
		After storage for 8 days									
B	11	0.5	*	*	*	*	*	*	*	*	*
C	12	4.5	*	225	*	*	*	*	*	*	*
B	13	0.6	*	1.7	*	*	*	40	*	*	0.8
A	14	200	0.3	75	39	*	*	*	*	6.8	*
B	15	340	*	15	*	*	*	*	*	*	*
		After storage for 16 days									
B	11	0.6	78	†	†	†	†	†	†	†	†
C	12	3	*	10	*	*	*	*	*	*	*
B	13	0.5	†	†	†	†	†	†	†	†	†
A	14	1600	*	†	†	†	†	†	†	†	†
B	15	2500	*	15	†	†	†	†	†	†	†

* = < 300 bacteria per ml.
† = > 3,000,000 bacteria per ml. (pin-point colonies too numerous to count).

be due to bacterial contamination of the samples during storage. That is, the tubes from which the subsamples for plate counts were taken were opened every 2 days during storage in order to make motility observations on the spermatozoa. Thus, it is possible that penicillin-resistant organisms were added to the samples. The bacterial counts on the yolk-citrate diluter stored for 8 days were the same, with only one exception, as those obtained prior to storage. These tubes were not opened during the 8-day storage period and thus contamination apparently was avoided.

Total plate counts on portions of freshly collected semen showed considerable variation, ranging from 200 to 960,000 bacteria per ml. with a mean of 364,000 bacteria per ml. for the five ejaculates studied. The semen samples were collected by means of an artificial vagina from three bulls used

TABLE 4

The stability of penicillin in diluted semen stored at 4.5° C. (mean of 9 determinations)

Theoretical units of penicillin*	Units of penicillin by assay (per ml. of diluted semen)		
	Before storage	After storage for	
		8 days	16 days
Control	0	0	0
250	266	270	233
500	592	568	468
750	857	815	719
1000	1133	1152	963
1250	1370	1313	1109
1500	1707	1718	1393
2000	2318	2156	1952
Diluter alone	0	0	0

* Number of units expected based on the total units in the ampules according to the producer.

for natural breeding in the College herd and no special precautions were taken to clean the bulls prior to collection.

Stability of penicillin in diluted semen. Penicillin assays were made on nine samples of diluted semen after storage for 0, 8, and 16 days, not only to determine the stability of the antibiotic but also to obtain more definite information on the actual number of units of penicillin added to the tubes of diluted semen. The latter was of particular interest since producers of penicillin usually add some excess units of penicillin to their ampules. Assays were made according to the standard cylinder plate method of Schmidt and Moyer (7). *Staphylococcus aureus* was the test organism employed. Plain yolk-citrate diluter was also assayed against this organism in order to ascertain whether or not it had any antibacterial activity.

The data in table 4 show that there was practically no loss of penicillin activity in diluted semen stored for 8 days at 4.5° C. Even after storage

for 16 days only a slight decrease in concentration occurred. The temperature employed, as well as the fact that apparently no coliform organisms were present in the samples, was undoubtedly responsible to a large extent for the results obtained. Organisms of the coliform group, particularly *Escherichia coli* (1), are capable of producing a penicillinase which destroys penicillin.

As indicated in table 4 the units of penicillin obtained by assay on portions of the diluted semen immediately following preparation always exceeded the theoretical number of units. Assays on yolk-citrate diluter showed that it possessed no antibiotic activity.

SUMMARY

1. The addition of 250, 500, and 750 Oxford units of penicillin per ml. of diluted semen did not significantly reduce the ability of spermatozoa to maintain motility during a storage period of 20 days. Levels of penicillin ranging from 1,000 to 2,000 units per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a 20-day storage period.

2. In routine artificial breeding, semen is seldom used after holding more than 6 days. When compared with untreated control samples, no significant decrease in maintenance of spermatozoan motility during a 6-day storage period occurred as the result of addition of 250, 500, 750, or 1,000 units of penicillin per ml. of diluted semen, but higher levels of penicillin were deleterious.

3. Within the limits of laboratory experiment, the relation between spermatozoan livability and level of penicillin is well represented by a straight line, the mean percentage of motile spermatozoa during storage for 20 days decreasing by 0.9 per cent for each additional 250 units of penicillin.

4. Penicillin depressed the utilization of glucose at all levels studied (250 to 2,000 units per ml. of diluted semen), while the amounts of lactic acid which accumulated were not significantly affected. Addition of penicillin increased the percentage of glucose utilized which was recovered as lactic acid.

5. Penicillin retarded bacterial growth at all levels in both freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was found in the semen without penicillin. The initial plate counts for the five ejaculates studied ranged from 200 to 960,000 bacteria per ml. of undiluted semen, with a mean of 364,000 bacteria per ml.

6. There was no appreciable loss in penicillin activity in diluted semen stored for 8 days and only a slight decrease in concentration after storage for 16 days at 4.5° C.

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THE EFFECT OF CONDITIONS OF STORAGE ON THE VISCOSITY OF SWEETENED CONDENSED MILK

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The viscosity of sweetened condensed milk increases with age until a gel structure develops. The time required for the milk to change from a fluid to a gel state depends on the quality of the raw milk, the manufacturing processes employed, the composition of the finished milk, and the conditions of storage to which the product is subjected. This paper is concerned with the effect on viscosity of variations in storage conditions, especially the time and temperature of storage.

Sweeetened condensed milk should be smooth and free-flowing, but viscous enough to prevent settling of lactose and rise of fat during storage. When age thickening proceeds to the stage of gelation, the milk is no longer suitable for many food uses. Deterioration in flavor generally accompanies the change in viscosity. The data in this report indicate some of the storage conditions that retard undesirable increases in viscosity.

EXPERIMENTAL PROCEDURE

The preparation of laboratory samples of sweetened condensed milk that were uniform in viscosity from batch to batch and from day to day was found to be very difficult. After extensive trials a technique was developed for the processing of 100 lb. of milk of 3.8 per cent fat and 9.15 per cent solids-not-fat, to which was added during concentration 18 lb. of sugar as a boiled sirup. All temperatures were controlled carefully, and the time required to perform each operation was the same for every batch of milk. The milk was forewarmed in a steam-jacketed kettle and concentrated in a 28-inch vacuum pan equipped with a steam jacket for finishing small batches of milk. Cooling and crystallizing were done in a water-jacketed vessel equipped with a stirrer.

Despite the precautions that were taken in preparing laboratory samples, these age thickened more rapidly than did the commercial milks. The laboratory samples were used chiefly in preliminary tests. The results obtained with the experimental milks differed from those with the commercial products in the magnitude of their viscosities, but the relationship between viscosity and the factors being studied was approximately the same in both types of samples.

The commercial sweetened condensed milk was prepared as parts of regular runs in a large condensery in northern New York. Grade A raw

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milk was used, and all manufacturing operations were conducted according to the best factory practice. The finished product was representative of the highest commerical grade of sweetened condensed milk. The condensed milk was shipped from the plant to the Bureau laboratories by express, the trip requiring 5 or 6 days.

The milks were stored in rooms where the temperature was controlled so that fluctuations did not exceed $\pm 2°$ F. Samples were not moved or disturbed during storage.

Reliable viscosity determinations on sweetened condensed milk are difficult to make. Stebnitz and Sommer (6) constructed a special viscosimeter utilizing the falling-sphere method. The measurements reported here were made under carefully controlled conditions with a McMichael viscosimeter and standardized wires. The determinations were made at 86° F. in a room maintained at this temperature. Each determination was made with a new sample of milk transferred in the same way and without stirring to the viscosimeter cup. Measurements often were made on duplicate samples and sometimes five or six samples were used to establish a single value. All milk samples either had an initial viscosity or soon developed a viscosity high enough to prevent lactose and fat separation during storage. It is believed that the viscosity values obtained during the work reflect the body condition the consumer would find in the milk. The viscosity is reported in poises but, because of the nature of the material, the values are relative rather than absolute.

Consideration was given to the possibility of bacterial growth during storage of the sweetened condensed milk. Rice and Downs (4) found that most of the organisms that might cause age thickening grew when the sugar ratio was less than 62.5 $\left(\text{sugar ratio} = \frac{\%\ \text{sugar}}{\%\ \text{sugar} + \%\ \text{water}} \times 100\right)$ but that growth sometimes occurred up to 64.5. The increase in titratable acidity found by Rice and Downs to accompany bacterial growth was 0.2 to 0.6 per cent after about 30 to 60 days of incubation.

The sugar ratio of the milks used in these experiments was 62.5 to 63.0. The titratable acidity of the samples stored at various temperatures was not always determined, but the tests that were made showed that the acidity of the milks increased about 0.1 per cent during a 3- or 4-month period at the higher temperatures. The age thickening observed in the sweetened condensed milks discussed in this paper was not considered to be influenced significantly by bacterial changes.

RESULTS

Cooling of the product and crystallization of the lactose in sweetened condensed milk may require 2 or 3 hours at temperatures that affect the viscosity of the milk. The effect of the rate of cooling sweetened condensed milk on

its age thickening is shown in table 1. The data represent the average values from five experiments with five different batches of milk. After the concentrates were dropped from the pan, they were cooled to 86° F. in 10 to 15 minutes and seeded. The cooling process of the rapidly cooled samples was continued, but the slowly cooled concentrates were held and stirred at 86° F. for about 2 hours before cooling was continued. The results indicate that the rate of age thickening is substantially the same for both methods of cooling. The lactose crystals in the rapidly cooled milk were a little larger (about 16 μ) in size than the lactose crystals in the slowly cooled milk (about 12 μ).

The relationship between container size and the age thickening of sweetened condensed milk was investigated. The experiments were done on fresh, commercially manufactured samples that were received in the Bureau

TABLE 1

*The effect of rate of cooling on age thickening of sweetened condensed milk**

Storage time at 86° F.	Viscosity after storage	
	Cooled in 162 min. to 63° F.	Cooled in 38 min. to 52° F.
(*days*)	(*poises*)	(*poises*)
1	115	110
4	204	193
12	365	362
24	583	550

* The data represent average values from 2 skim and 3 whole sweetened condensed milks. The samples were made in the Research Laboratory pilot plant during March and April. After condensation, each batch was divided into two parts, one for slow and the other for rapid cooling. The cooled milk was canned and placed at once in a storage room at 86° F.

laboratories in 30-gallon tight oak barrels. Smaller containers in the form of cans of various sizes were filled with sweetened condensed milk. About 3 gallons of milk was taken from the test barrels for this purpose. During the storage period one of the small cans of milk was opened for each viscosity determination.

The milk in the barrels was sampled through the bungs by means of a 1-inch diameter metal tube inserted diagonally from the bung toward one end of the barrel. Care was taken to close the bungs tightly after sampling. Table 2 shows some results of the study of the effect of container size on the viscosity of sweetened condensed milk. The tight oak barrels of sweetened condensed milk were held on the bilge with bungs down.

Two barrels of the milk received in September, 1944, were put in storage at 86° F. Samples were obtained from one barrel while the other was held unopened until the end of the test. When the sealed barrel was opened at the bung after 76 days, there was insufficient oxygen in the headspace to

support a match flame. The average viscosity of the milk (380 poises) and the titratable acidity (0.55 per cent) were substantially the same as those of the milk in the barrel that had been used for sampling (table 2).

The viscosity of the milk in the barrel that was not opened for sampling was different in various parts of the barrel, after 76 days at 86° F., being 517 poises at the surface and 478 poises at the bottom. In several places near the center of the barrel the viscosity of the milk was 352 poises. When the barrel was filled by the manufacturer, the temperature of the milk was about 50° F., and the milk in the center of the barrel probably required appreciable time to warm to 86° F. In addition, some surface thickening

TABLE 2

Effect of container size on the viscosity of two lots of sweetened condensed milk of commercial manufacture

Storage time	Barrel (30 gal.)	No. 3 can	No. 1 can	Baby can
(*days*)	(*poises*)	(*poises*)	(*poises*)	(*poises*)
Milk of September 27, 1943*—storage temperature 70° F.				
1	69	.....	69	69
22	70	.....	78	81
40	92	.....	111	115
92	140	.....	.....	182
180	148	.....	233	255
256	246	.....	285	346
357	368	.....	446	550
Milk of September 26, 1944†—storage temperature 86° F.				
1	39	39	39	39
8	64	88	86	86
17	80	101	108	119
35	143	168	176	176
74	385	407	429	429

Composition figures furnished by the manufacturer:
* 9.25% fat, 23.0% M.S.N.F., 42.75% sucrose, 25% water.
† 8.73% fat, 23.62% M.S.N.F., 42.75% sucrose, 24.9% water.
Sugar ratio of these milks = 63.

may have been caused when this barrel dried and leaked slightly between the staves. This occurred in the middle of the storage period but the staves tightened up in 3 days when the humidity in the storage room was raised.

The effect of time and temperature of storage upon the viscosity of sweetened condensed milk was investigated. Samples were prepared June 1, 1945, by a cooperating manufacturer as part of a large commercial batch. The samples contained 9.61 per cent fat, 23.91 per cent M.S.N.F., 41.65 per cent sugar, and 24.83 per cent moisture. A barrel of this sweetened condensed milk was shipped to a canning plant, where it was re-packed in special 2-ounce cans on June 8. Four hundred of these small cans were received in these laboratories June 21 and stored at six different tempera-

tures. Each can held enough milk to make one viscosity determination. Some of the results are plotted in figure 1.

This milk showed a large increase in acidity with age, but apparently this was not caused by bacterial growth. The figures determined on the manufacturer's milk in six of the 2-ounce cans showed an average standard plate count of 4,380 and a titratable acidity of 0.43 per cent. After 640

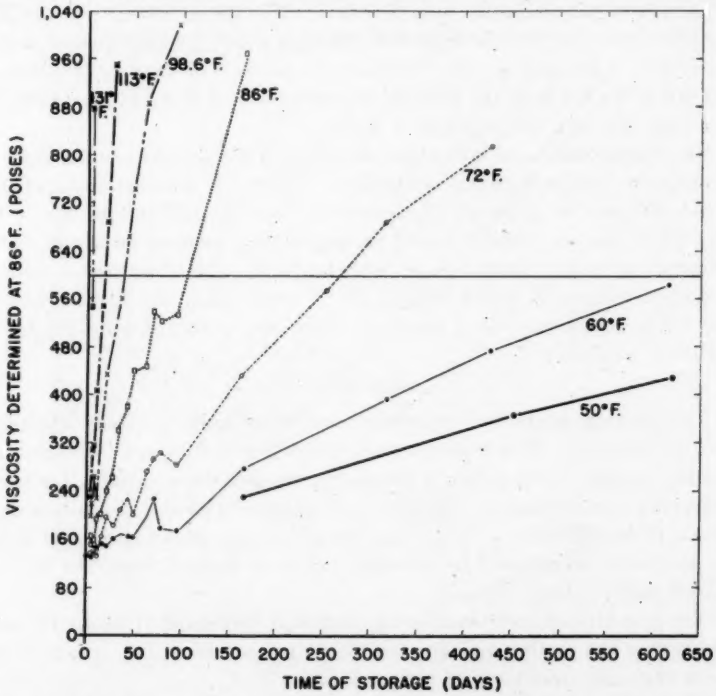

FIG. 1. The effect of time and temperature of storage on the viscosity of sweetened condensed milk stored in 2-ounce cans. All samples were from the same batch of milk, but the samples used for storage at 50° F. were delayed 2 weeks in transit at summer temperatures, causing an undue initial increase in viscosity. The composition of the milk was 9.61 per cent fat, 23.91 per cent M.S.N.F., 41.65 per cent sugar, and 24.83 per cent water.

days of storage at different temperatures, the titratable acidity and the acid intensity of this milk were: 50° F. storage, 0.84 per cent and pH 6.01; 60° F. storage, 0.85 per cent and pH 6.09; 72° F. storage, 1.0 per cent and pH 5.90. No signs of bacterial growth were found in samples held at these temperatures for 640 days.[1] Anaerobe tubes, direct smears, and standard

[1] Bacteriological examination of this milk was made by Harold R. Curran of these laboratories.

plates were practically negative. While it was not shown that growth of organisms was absent during the whole storage period, the available evidence indicates that the acidity increase was due to chemical changes rather than to bacterial activity.

The storage temperature of a group of the 2-ounce cans of sweetened condensed milk was varied from 60° F. to 98° F. by shifting the cans every 24 hours from one temperature to the other. The initial viscosity of the milk was 132 poises, but after storage for 170 days a soft gel had formed with a viscosity of 1,155 poises. By reference to figure 1 it may be determined that this is the viscosity the milk would have reached if it had been held 170 days at a constant temperature of 89° F.

Sweetened condensed milks that were stored for several months at 0° F. did not show important physical changes. There was no measurable change in viscosity and no apparent change in the dispersion of the protein or the fat. There was no protein flaking or insolubility, such as occurs in frozen unsweetened milk. There was an increase in the size of lactose crystals in sweetened condensed milks held at 0° F. only when the lactose was incompletely crystallized as a result of improper cooling during the manufacturing process.

DISCUSSION

The keeping quality of sweetened condensed milk is closely associated with its viscosity. Whenever sweetened condensed milk age thickened to a viscosity of 600 to 800 poises, it generally was no longer suitable for use in high-grade food products. Off flavors often developed and the milk was too viscous to handle easily. A soft gel structure was present at 1,000 poises. The gel could be reduced by stirring, but it re-formed when the milk remained undisturbed.

The viscosity of sweetened condensed milk increased at about the same rate during the cooling and crystallization periods as it did during storage under the same conditions of temperature.

The data of table 2 indicate that sweetened condensed milk packed in barrels will remain fluid a little longer than milk packed in cans when both are held under the same conditions of storage. The temperature of milk in cans follows fluctuations in storage temperature more closely than does the temperature of milk in barrels. Commercial experience indicates that the sweetened condensed milk in the middle of a 50-gallon barrel will require about 7 days to reach 85° F. after previous storage at about 60° F. (1). A longer time is required for the reverse change to take place. Cool milk packed in barrels will remain cool and thin longer after the container is placed at a high temperature than will milk in small cans.

These considerations, and the fact that high sugar concentrations stabilize the milk and retard viscosity changes, support the suggestion (2)

that bulk sweetened condensed milk may have a minimum sugar ratio of 60, while the sugar ratio of the canned milk should be at least 62.5. However, a bulk sweetened condensed milk with a sugar ratio of 62.5 will be superior to one that contains less sugar.

Data from the curves of figure 1 were used to prepare figure 2, in which the logarithms of the viscosities are plotted against the temperatures of storage.

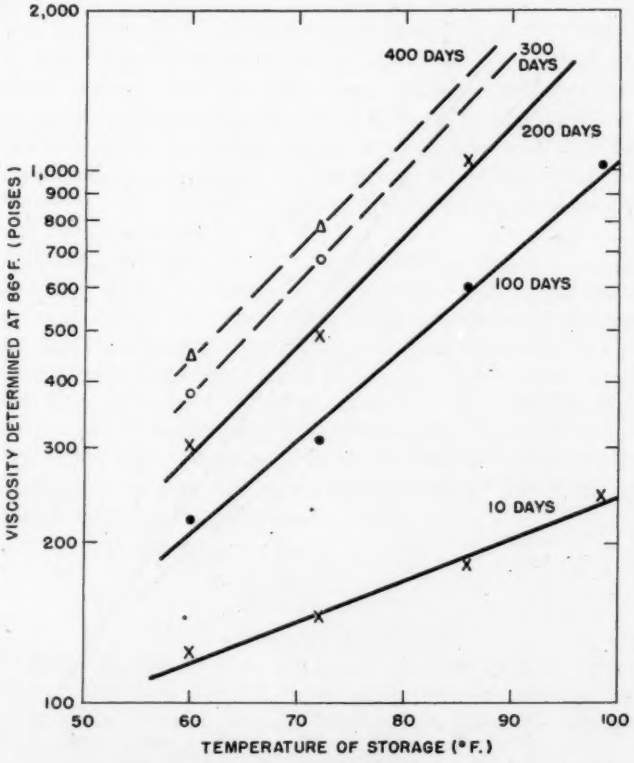

FIG. 2. The effect of time and temperature of storage on the viscosity of sweetened condensed milk. Data taken from figure 1.

The time required for the sweetened condensed milk to reach a viscosity of 600 poises at different temperatures is indicated in figure 1. When these temperature data were plotted against the logarithms of the time of storage, straight line no. 1, figure 3, was obtained. Values for 30, 40, and 50° F., obtained by extrapolation of curve no. 1, figure 3, are 4,950, 2,460, and 1,225 days, respectively. Other data also are plotted on figure 3.

If n = viscosity and c, c', c'' = constants, the relationship shown in figure 1 may be expressed as follows: $n = c'$ (time of storage) or $\log n = c''$ (log time), while for figure 2 $\log n = c$ (temperature of storage); then c (temperature) $= c''$ (log time), the relationship shown in figure 3 where the viscosity is constant.

The data given in figure 1 and re-plotted in figures 2 and 3 show that

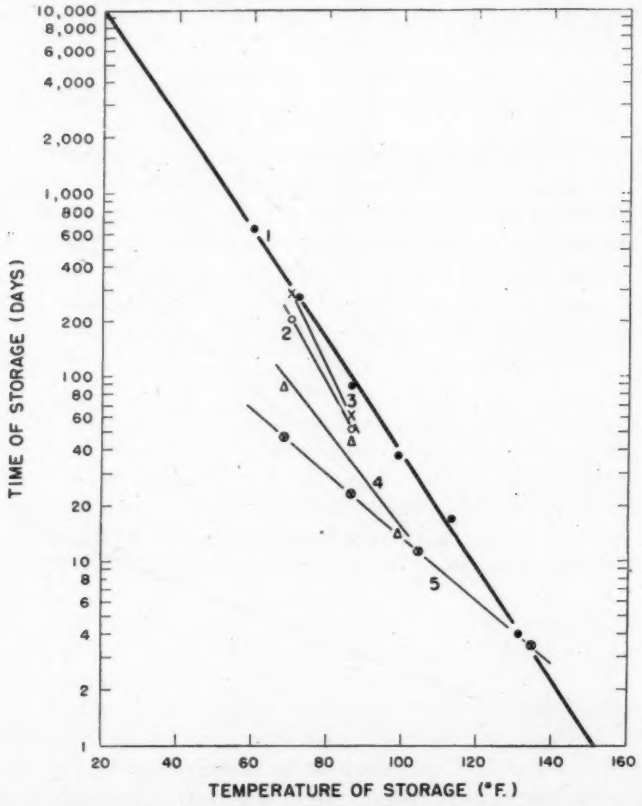

FIG. 3. The relationship between the time and temperature of storage in the development of viscosity of sweetened condensed milk: Curve 1, data from figure 1, viscosity of 600 poises. Curve 2, data from table 2, viscosity of 300 poises. The points for 70° F. and for 86° F. were obtained with different milks stored in baby-size cans. Curve 3, data from table 2, viscosity equals 300 poises. The points for 70° F. and for 86° F. were obtained with different milks stored in barrels. Curve 4, data from Rogers *et al.* (5), viscosity of 12° rotation on special viscosimeter. Curve 5, data from Leighton and Mudge (3) in which the time is given in hours (not days) required for the milk to start thickening.

the viscosity of sweetened condensed milk increases arithmetically with time of storage but logarithmically with temperature of storage. For conditions of constant viscosity the time of storage varies logarithmically with the temperature of storage.

Although the logarithms of the storage times required for a sweetened condensed milk to reach a certain viscosity, plotted against the temperatures of storage, yield a straight line (fig. 3), the slope of this line may vary with different milks. The milk used by Leighton and Mudge (3), figure 3, gave a line of different slope from that of the other milks. The data of Leighton and Mudge were obtained by observing the number of hours required for the milk to start thickening. It seems probable that most commercial milks will thicken much as did milk no. 1 of figure 3. It should be possible to estimate the length of time a sweetened condensed milk will retain a satisfactory viscosity at various storage temperatures by using the data of figure 3. The rate of thickening approximately doubles with each increase of 10° F. between 30 and 60° F.

In some cases the slope of the time-temperature curve of a milk may differ from that of curve 1, figure 3. A line may be drawn for any milk if two points are obtained. To secure these points quickly, about six samples of a milk may be held at each of two high temperatures such as 98° and 120° F. Viscosity determinations should be made at 1- or 2-day intervals and curves drawn like those of figure 1. Two points having the same viscosity value then may be used to construct a time-temperature curve patterned after that of figure 3. Other points may be taken from the curve or they may be calculated from the geometric equation for a straight line. The equation may be stated in the following form:

$$\frac{\log y - \log y_1}{\log y_2 - \log y_1} = \frac{x - x_1}{x_2 - x_1}$$

when

y = storage time

x = storage temperature

y_1 and y_2 = storage times at x_1 and x_2 storage temperatures. For a milk similar to that shown in curve 1, figure 3, the storage time (y) required to reach a viscosity of 600 poises at a given temperature (x) may be found from the equation calculated from the plot:

$$\log y = 4.64 - \frac{1.82\ x}{59}.$$

It is important to note that the time-temperature relationship of figure 3 will hold only when the storage temperature is constant. If the storage temperature fluctuates, the changes in viscosity will be dependent upon the extent of the changes.

The theoretical freezing point of sweetened condensed milk is about 5° F., but only a few ice crystals form at this temperature. Since all the

moisture in sweetened condensed milk will freeeze only at a much lower temperature, neither the milk nor the container is damaged by storage at temperatures considerably below 0° F. Sweetened condensed milk that was held at 0° F. for many months did not change measurably in viscosity. Extrapolation of Curve 1, figure 3, indicates that 24,000 days (65 years) at 0° F. would be required for this milk to reach a viscosity of 600 poises.

SUMMARY

The viscosities of sweetened condensed milks held in the same storage room increased a little more rapidly in the milks packed in small cans than they did in the milks packed in barrels.

The viscosity of sweetened condensed milk increased logarithmically with increases in storage temperature and arithmetically with increases in storage time. For conditions of constant viscosity, time varied logarithmically with temperature. Viscosity values may be predicted by applying this relationship to time-temperature data from high temperature, accelerated storage tests.

The viscosity of sweetened condensed milk increased at about the same rate during the cooling and crystallization periods as it did during storage under identical conditions of time and temperature.

The authors wish to express their appreciation to P. L. Haymes, H. D. Wilder, and A. R. Davis, of the United Milk Products Company, for their numerous suggestions and their active interest in this project and for their manufacture of the commercial samples of sweetened condensed milk.

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DETERMINATION OF CHEESE LIPASE[1]

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In recent years lipolysis has been directly associated with flavor development in Cheddar cheese. In order to study directly the fat breakdown in Cheddar cheese, procedures for the quantitative determination of the lipolytic agents responsible are necessary.

A method for the determination of milk lipase previously was reported by the present authors (1). Since no methods for the quantitative determination of cheese lipase have been described, it was necessary to devise one. In the present paper such a method is presented. It is similar in principle to the milk lipase determination previously reported (1) and, similarly, has the limitation that it measures only enzymes capable of hydrolyzing tributyrin.

EXPERIMENTAL

Effect of pH on cheese lipase activity. In figure 1 the pH activity curve for the hydrolysis of tributyrin by cheese extracts is given. Determinations of enzyme activity at various pH values were carried out according to the procedure subsequently outlined under "Quantitative Determination of Cheese Lipase" with the following exception: In place of 2 ml. of 0.38 molar aniline buffer, 2 ml. of a 0.38 molar aniline, 0.38 molar phosphate buffer were added. By means of this composite buffer, pH was controlled throughout the pH range desired.

It will be seen from the figure that tributyrin is split most rapidly at pH 5. There also is a secondary optimum at pH 6.5 to 7.0. This secondary pH optimum seems to vary with the age of the cheese. The primary pH optimum of the lipolytic activity seems quite constant, however, throughout the cheese-ripening period. The presence of two peaks in the pH optimum curve of cheese lipase indicates strongly that two or more lipases are present. The enzymes active at pH 6.5, however, are not active in ripening cheese, since the pH of the cheese seldom rises above 5.4. Therefore, only the lipolytic activity at pH 5 was considered important.

Effect of buffers on cheese lipase activity. In the development of a method for the estimation of cheese lipase, it was necessary to find a buffer which did not affect the lipase activity and which had good buffering capacity in the desired pH range. In table 1 are shown the effects of six buffers upon the activity of cheese lipase. As may be seen, the citrate, acetate, and

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phthalate buffers had an inhibitory effect on the lipase. None of the other three buffers inhibited the enzyme. Any buffer containing the carboxyl group seems to inhibit the lipase. The flattening of the curves in figures 3 and 4 when a titration greater than 0.40 ml. of 0.1 N NaOH is obtained per 5 ml. of incubation mixture probably is due to reversible combination of the butyric acid with the enzyme to form an enzyme-product complex. The buffers containing carboxyl groups probably inhibit the lipase in the same way. The phosphate buffer was not used in the cheese lipase method as

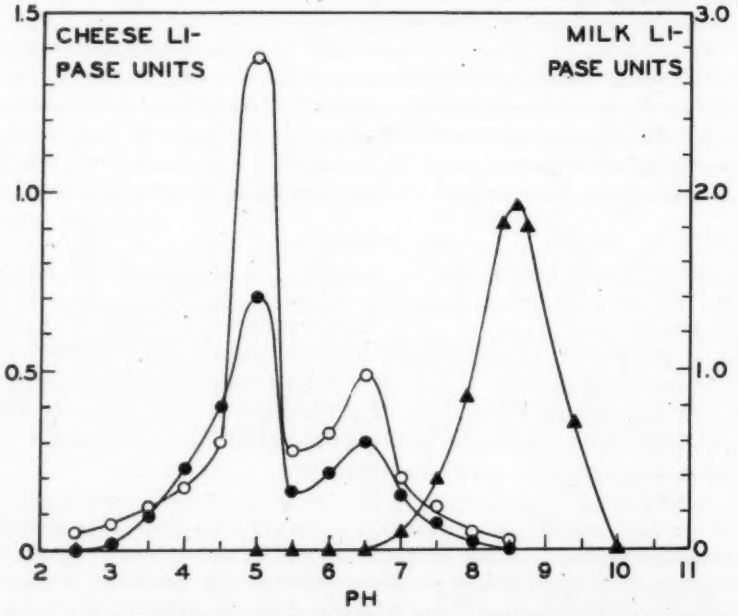

FIG. 1. Effect of pH on rate of tributyrin hydrolysis by cheese extracts. The effect of pH on the rate of tributyrin hydrolysis by milk lipase is presented for comparison. Triangles represent milk lipase, dots represent 2-month-old raw milk cheese, and circles represent 10-month-old raw milk cheese.

finally adopted because it has very little buffering capacity at pH 5. Both the pyridine and aniline buffers have good buffering capacity at pH 5. Since the aniline buffer was the more convenient to use, it was chosen for use in the final method.

Effect of activators on cheese lipase activity. Various compounds known to possess definite activating properties for other enzymes were tested for activating effect on cheese lipase. As may be seen in table 1, none of them gave activation; most of them were inhibitors.

TABLE 1

The effect of activators and buffers on cheese lipase activity

Substance used	Concentration	Apparent cheese lipase in sample
	Activators	*(units/ml.)*
None	.....	0.88
Zinc chloride	0.001	0.82
Potassium cyanide	0.001	0.64
Manganous sulphate	0.001	0.80
Magnesium chloride	0.001	0.88
Calcium chloride	0.001	0.90
Cysteine	0.001	0.78
	Buffers	
Aniline	0.0625	0.70*
Pyridine	0.1	0.70
Phosphate	0.1	0.70
Citrate	0.1	0.16
Phthalate	0.1	0.22
Acetate	0.1	0.34

* The lipase solution used in the buffer experiments was not the same as that used in the activator experiments.

Variation of hydrolysis rate with amount of tributyrin present. Figure 2 shows that the rate of hydrolysis of tributyrin by cheese lipase is affected by concentrations of tributyrin up to 1.5 per cent. At this level and above, the hydrolysis rate appears to be constant.

Kinetics of cheese lipase. In figure 3 the relation between quantity of cheese lipase and tributyrin hydrolysis is presented. It may be seen that the tributyrin hydrolysis obtained is directly proportional to the quantity of cheese lipase present, up to the point when 0.40 ml. of 0.1 N butyric acid is present in 5 ml. of incubation mixture.

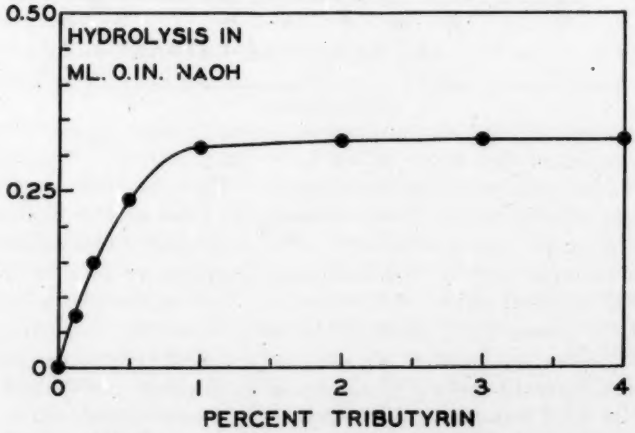

FIG. 2. Variation of hydrolysis rate with amount of tributyrin present.

In figure 4 the effect of incubation time on tributyrin hydrolysis by cheese lipase is shown. It may be seen that the hydrolysis of tributyrin by cheese lipase at pH 5 is linear up to the time when 0.40 ml. of 0.1 N butyric acid is present in 5 ml. of incubation mixture.

The enzyme unit used in the cheese lipase determination is based on the relationships presented in figure 3.

QUANTITATIVE DETERMINATION OF CHEESE LIPASE

In the method finally adopted, the following procedure is used. Twenty grams of the cheese sample to be tested are weighed out with an accuracy of $\pm$ 0.1 g. To the weighed sample in a Waring Blendor or other suitable

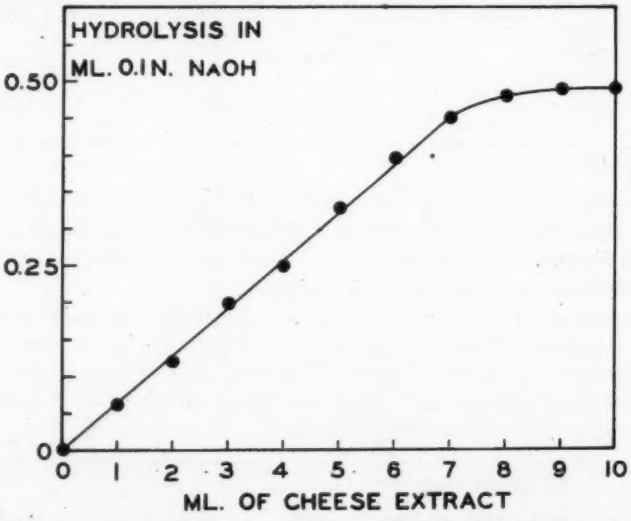

FIG. 3. Relation between quantity of cheese lipase and amount of tributyrin hydrolysis.

mixer, enough distilled water is added to make a total volume of 100 ml. The power mixer then is run at low speed for 2 minutes. This length of time is ample to macerate the cheese sample. The mixer then is set to high speed and allowed to run 7 more minutes. At the end of this time the mixture is in the form of a smooth, white suspension. This suspension is transferred quantitatively to a small hand homogenizer using a minimum amount of distilled water. The suspension then is run five to six times through the homogenizer, which further insures complete extraction of the enzyme. After quantitative transfer to a 250-ml. centrifuge tube, the suspension is centrifuged for 10 minutes at 2,000 r.p.m. At the end of this period the solid material in the suspension will have settled out, leaving a faintly turbid supernatant which is drawn off. The residue is washed care-

fully and centrifuged at 2,000 r.p.m. for 10 minutes with three successive 20 ml. portions of distilled water, which all subsequently are added to the turbid supernatant. The supernatant plus the three washings is diluted to a total volume of 200 ml. and the resulting cheese extract is used directly as the enzyme sample to be analyzed. Experiments have shown that all the lipolytic activity present in the cheese is washed free from the cheese curd by the above procedure.

For the analysis, a suitable volume (depending upon its activity) of the

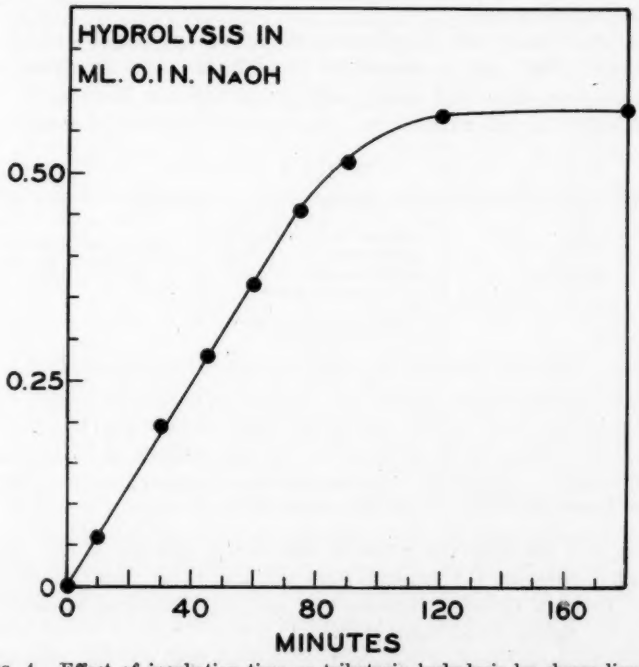

FIG. 4. Effect of incubation time on tributyrin hydrolysis by cheese lipase.

cheese extract is added to 2 ml. of 0.38 molar aniline buffer at pH 5. This solution is diluted to a volume of 12 ml. with distilled water and placed in a water bath held at 40° C. After the solution has attained this temperature, 0.2 ml. of tributyrin is added, making a total volume of 12.2 ml. The mixture is shaken vigorously for 1 minute, and a 5-ml. aliquot immediately is removed for titration. After an incubation period of 60 minutes[2] at 40° C., the tube and its contents are shaken for 30 seconds and another 5-ml. aliquot is titrated. The difference in titration between the two aliquots

[2] In all the experimental work presented, incubation times of 60 minutes were used unless otherwise specified.

represents tributyrin hydrolysis. The titration is carried out as follows: The 5-ml. aliquot is pipetted into a 50-ml. Erlenmeyer flask containing 5 ml. of a 0.02 per cent solution of thymolphthalein in 95 per cent alcohol. After addition of 2 ml. of ether, the solution is titrated with 0.1 N alcoholic NaOH from a burette calibrated at 0.01-ml. intervals. Titration is carried to a definite blue color. It is convenient to prepare an artificial endpoint comparison flask containing a dilute aqueous solution of $CuSO_4$ and $CoCl_2$ to which enough alumina cream has been added to give a close resemblance to the actual titration flask.

One cheese lipase unit is defined as the amount of enzyme which, when diluted to 12.2 ml. and incubated for 60 minutes under conditions of the determination as described above, will give a titration increase of 0.1 ml. of 0.1 N NaOH for a 5-ml. aliquot. For instance, if 4 ml. of cheese extract

TABLE 2

Comparison of volatile acid distillate titration differences and direct titration differences

Enzyme sample no.	Amount of hydrolysis of tributyrin by titration differences of volatile acid distillates	Amount of hydrolysis of tributyrin by direct titration differences
	*(ml. 0.1 N NaOH)**	*(ml. 0.1 N NaOH)**
A–1	0.05	0.05
A–2	0.36	0.34
A–3	0.42	0.43
A–4	0.17	0.19
A–5	0.27	0.27
A–6	0.12	0.11

* All values expressed as ml. 0.1 N NaOH represent the amount of hydrolysis of tributyrin by various dilutions of cheese enzyme sample A.

are used, and the observed titration increase is 0.36 ml. of 0.1 N NaOH, the extract contains 0.9 enzyme unit per ml. The linear relation here assumed between titration increase and amount of enzyme is shown to be justified by figure 3 and table 3.

If the observed titration increase is more than 0.40 ml., the determination should be repeated with a smaller volume of cheese extract. If the titration increase is less than 0.1 ml., a larger volume should be used. Cheese extracts have been found to retain their activity for at least 10 hours in the refrigerator.

RELIABILITY OF THE METHOD

Evidence that the titration used measures only glyceride hydrolysis is afforded by table 2, where direct titration figures are compared with titrations of volatile acid distillates of equivalent aliquots. Good agreement was obtained in all cases. The volatile acid distillates were obtained as follows: A 5-ml. aliquot of the incubation mixture was pipetted into the distillation

flask. The pH immediately was adjusted to 2 (red to thymol blue) with 5 N H_2SO_4. A constant volume distillation then was carried out until about five times the volume of the original sample had been distilled over. The distillates obtained were titrated to the phenol red endpoint.

The reliability of the method and of the standard curve also was checked by running varying concentrations of the same enzyme preparation. The results may be found in table 3. As may be seen, determinations agreed within 10 per cent.

TABLE 3

Effect of sample size on apparent cheese lipase content of a cheese extract

Sample size	Titration differences	Lipase content
(ml.)	*(ml. 0.1 N NaOH)*	*(units/ml.)*
1.0	0.07	0.70
1.0	0.08	0.80
3.0	0.23	0.77
3.0	0.23	0.77
5.0	0.38	0.76
5.0	0.39	0.78
6.0	0.45	0.75
6.0	0.46	0.77
7.0	0.47	0.67

DISCUSSION

It should be emphasized that the method outlined here has a number of failings. Since the lipolytic activity of Cheddar cheese undoubtedly is due to a mixture of lipases produced by the mixed flora of the cheese, any method of quantitative estimation will give consistent results only if the ratio in which the various lipases are present remains unchanged. This is true because various substrates are attacked at different rates by different enzymes. If a mixed substrate such as butterfat is used, the determination does not inform us whether the fatty acids liberated are the highly flavored volatile fatty acids or the relatively tasteless higher fatty acids. On the other hand, if a pure substance is used as a substrate, the determination can measure only activity toward this substrate, and again no direct information is obtained regarding liberation of the particular acids of interest to the investigator.

Any lipase determination will measure only lipases active at the pH of the determination. The present determination will not detect the presence of lipases active only at high pH values, since the determination is designed to measure only those enzymes active at pH 5.

SUMMARY

1. A method is presented for the quantitative determination of lipolytic activity in cheese. Determinations run at various sample levels agree within 10 per cent.

2. Hydrolysis of tributyrin by the Cheddar cheese lipase system is most rapid at pH 5. A secondary optimum occurs at pH 6.5 to 7.0.

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LIPASE ACTIVITY DURING MAKING AND RIPENING OF CHEDDAR CHEESE[1]

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In recent years it often has been assumed that rancidity in cheese is due to the production of lower fatty acids such as butyric acid by the action of milk lipase on natural milk fats. It first was suggested by Rice and Markley (18) that milk lipase was one of the causes of rancidity in cheese. Lane and Hammer found that: (a) typical rancid Cheddar cheese could be produced experimentally by the addition of sources of lipase, such as rennet paste (11) and pancreatin (13), to cheese milk, and (b) typical rancid Cheddar cheese could be produced experimentally by the addition of homogenized raw cream to raw skim milk as a means of milk lipase activation (12). Hood *et al.* (10) confirmed the reports of Lane and Hammer and further found that typical rancid Cheddar cheese could be produced experimentally under commercial conditions by vigorous agitation of raw cheese milk at various temperatures (7, 8). Since these earlier publications, Hlynka and Hood (2, 3), Hlynka *et al.* (5, 6, 9), and others (1, 14) have reported a great deal of work which has been carried out on the basis of the milk lipase theory. A large part of this work has been carried out with commercial lipases or crude enzyme preparations as the source of experimental lipase. There is no reason to believe, however, that the lipases from these sources have properties comparable to milk lipase. Moreover, commercial lipase preparations and crude enzyme preparations generally contain many other types of enzymes as impurities.

In a brief preliminary report (15) Peterson and Johnson pointed out that milk lipase is inactive at the pH of Cheddar cheese and is completely absent from Cheddar cheese after pressing. The data on which these conclusions were based are presented in this paper. In a recent paper, Hlynka and Hood (4) also concluded that milk lipase is inactive in Cheddar cheese after it is made.

The purpose of the present paper is to present studies of the differences in cheese lipase content during the making and ripening of raw and pasteurized Cheddar cheese from the same milk. In the preliminary report (15) it was mentioned that lipolytic activity of a type different from that of milk lipase gradually appears in ripening Cheddar cheese. This

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lipolytic activity has been termed "cheese lipase" by the present authors (17). Cheese lipase is distinctly different from milk lipase in its pH optimum (17) and its chemical kinetics with relation to the hydrolysis of tributyrin (16, 17).

METHODS

Milk lipase. The method for the determination of milk lipase previously reported by the present authors (16) was used for all milk lipase analyses. In this method, the degree of hydrolysis of tributyrin at 40° C. by milk lipase in a solution buffered at pH 8.5 is used as a measure of milk lipase activity.

Cheese lipase. The method for the determination of cheese lipase reported by the present authors (17) was used for all cheese lipase analyses. In this method the degree of hydrolysis of tributyrin at 40° C. by cheese lipase in a solution buffered at pH 5.0 is used as a measure of cheese lipase activity.

Cheesemaking procedure. The milk used in making the experimental cheeses was mixed, raw, whole milk from the University Dairy. Small vats of cheese were made (460 lb. of milk). Each such vat yielded two 22-lb. cheeses. Cheeses were made by an experienced maker according to standard procedures. The green cheeses produced were kept at 68° F. for a period of 2–3 days and then were transferred to a cold room (40–45° F.) for the remainder of the ripening period.

To make both raw and pasteurized milk cheese from the same milk, 920 lb. of mixed, raw, whole milk was divided into two equal portions. The first portion received no treatment. The second portion was pasteurized by holding 30 minutes at 145° F. Each portion then was placed in a separate vat, and both received identical subsequent treatment according to standard Cheddar cheesemaking procedures.

A. ROLE OF MILK LIPASE IN CHEDDAR CHEESE

Loss of milk lipase during making. In table 1 the stability of milk lipase at various temperatures and pH values is presented. From these data it would be expected that under the pH and temperature conditions used in the making and ripening of Cheddar cheese (table 2), there is little possibility of milk lipase being present in green or ripe Cheddar cheese. Assuming it was present, milk lipase is inactive at a pH of 6.5 or lower (16, 17). Therefore, at the pH of ripening Cheddar cheese (5.0–5.4), milk lipase would have no effect even if present.

In order to obtain direct substantiation for the above conclusions, milk lipase estimations were made before, after, and, if necessary, during any operation in the making and ripening of raw milk Cheddar cheese that might affect the milk lipase content. A number of such experiments were

TABLE 1

Stability of milk lipase at various pH values

pH	Per cent of lipolytic activity remaining after 30 min. incubation		
	(*41° F.*)	(*68° F.*)	(*104° F.*)
4.0	58.2	14.4	0
4.5	72.7	41.0	0
5.0	88.1	48.0	8.1
5.5	99.1	72.3	18.2
6.0	99.1	97.1	44.8
6.5	99.2	98.9	92.0

carried out. The results of one representative experiment are presented in table 2.

As may be seen from the table, no significant change in milk lipase content takes place until the rennet is added to the raw milk. A rapid rise in milk lipase content immediately results. The cause of this rise is not definitely known since no lipolytic activity at pH 8.5 is present in rennet

TABLE 2

Role of milk lipase in Cheddar cheesemaking

Time	Temperature	pH	Source of sample	Milk lipase content*
(*min.*)	(° *F.*)			
0	55–57	6.60	Raw milk after addition to vat	29.0
13	86	6.60	Raw milk after warming to 30° C.	27.8
18	88	6.59	Raw milk after addition of starter†	27.8
73	88	6.59	Raw milk after addition of rennet‡	41.5
101	88	6.51	Coagulum after cutting of the curd	25.2
110	88		Coagulum before heating	24.0
133	104	6.48	Curd after heating to 104° F.	16.4
188	96	6.29	Curd during firming process	7.63
208	92	6.04	Curd just after dipping	1.36
208	91	6.12	Whey just after dipping	5.11
368	91	5.46	Curd just after milling	0.78
(*hr.*)				
24	Room temp.	5.31	Cheese just after pressing	0.00
(*days*)				
5	40–45	5.28	Cheese	0.00
10	40–45	5.27	Cheese	0.00

* Milk lipase content expressed in all cases as the number of milk lipase units per gram of cheese. All figures are based on the amount of cheese obtained, which equaled 9.72 lb. per 100 lb. of milk.

† One per cent inoculum of an 18-hr. commercial starter culture added.

‡ Commercial rennet added in the amount of 10 ml. per 100 lb. milk.

alone. Since this apparent milk lipase activity disappears rapidly, it is readily seen that the factor responsible is quite unstable. There are two important inactivations or removals of true milk lipase activity. The first takes place during the heating of the coagulum to 104° F. and the holding between this temperature and 96° F. for 55 minutes. After this treatment less than one-third of the original milk lipase activity remains. This is due not only to the temperature effects but also to the low pH range encountered (6.0–6.5). The combination of these two adverse factors is more effective in the inactivation of milk lipase than either one alone. Another large loss of milk lipase occurs during the removal of whey. After dipping, less than 5 per cent of the original milk lipase remains in the curd. Only a small amount of milk lipase remains in the cheese curd just after the milling operation. Due to the low pH (5.3–5.5) of the curd, this small amount of milk lipase is rapidly inactivated, and no milk lipase activity can be found in the green cheese 24 hours after the start of making. As may be seen from the table, analyses were also run after 5 and 10 days of ripening. No milk lipase activity was found.

Discussion. There probably are a number of lipases present in milk. It is very probable that the present method of measuring milk lipase activity does not include all of them. It also is probable, however, that those lipases present in milk which are not capable of splitting tributyrin have little or no effect on the production from milk fat of those fatty acids which could contribute to the flavor of Cheddar cheese. The fatty acids contributing to cheese flavor probably include only those having from two to ten carbon atoms. The fatty acids containing more than ten carbon atoms have little or no taste. Since the fatty acids capable of contributing flavor to cheese must lie within this narrow range of possible number of carbon atoms, there is no reason to believe that a lipase exists in milk capable of splitting, for example, caprylic acid but not butyric acid from a glyceride ester.

As far as rancidity in cheese is concerned, there is no doubt that tributryin is the most logical choice for a substrate in the measurement of milk lipases possibly causing the rancidity. This is true since rancidity in cheese generally is attributed to the production of lower fatty acids such as butyric acid. There is, of course, the remote possibility that a lipase present in milk would release butyric acid from mixed triglycerides and not from simple triglycerides.

B. LIPASE CONTENT OF CHEDDAR CHEESE AFTER MAKING

Lipase content of raw and pasteurized Cheddar cheese. After a ripening period of approximately 5–20 days, lipolytic activity of the cheese lipase type is found in green Cheddar cheese. In figure 1 the averages of the lipolytic activities of six pairs of pasteurized milk cheeses and raw milk

cheeses made by the procedure described under "Methods" are given. The average per cent deviation of the individual lipase analyses from the mean was less than 3 per cent.

The lipolytic activity in raw milk Cheddar cheese will be considered first. In figure 1 it will be noted that no lipolytic activity at pH 5 is present in the milk at the time it is placed in the vat. This is in accordance with data presented previously (16, 17) showing that milk lipase is inactive below pH 6.5. The first increase in lipolytic activity occurs at the time of addition of the rennet, which is about 75 minutes after the start of making. Since there is a similar increase in the lipolytic activity of corresponding

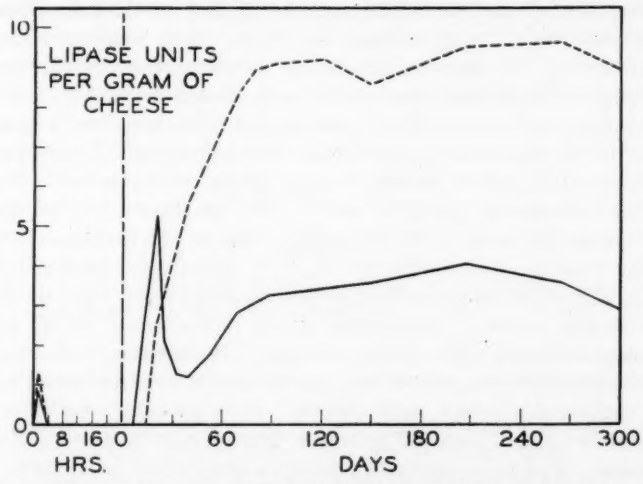

FIG. 1. Lipase activity at pH 5 in Cheddar cheese during making and a 300-day ripening period. Average values for six pairs of cheese. Solid line, pasteurized milk cheeses; broken line, raw milk cheeses.

pasteurized milk cheeses, undoubtedly this effect is caused by lipolytic enzymes which are active at pH 5 and are present in the rennet as impurities. Since this lipolytic activity attributable to rennet disappears at the time of dipping, it can have no effect on the cheese during the ripening period. After dipping there is no lipolytic activity present until after the cheese is in its final form and has been stored 5–20 days. At this time lipolytic activity is detectable, and it increases in amount steadily up to 80–90 days after making. Lipolytic activity in pasteurized milk Cheddar cheese can be detected in large amounts at an earlier stage in the ripening period than in raw milk Cheddar cheese. The significance of this will be discussed later.

Figure 1 shows that the amount of lipolytic activity at pH 5 present during the making of the cheese is very small compared to the lipolytic

activity at pH 5 of the cheese during the ripening period. Therefore, even if this lipolytic activity at pH 5 present during making is carried over into the cheese in a reversibly inactivated form, its role in ripening must be small.

At most a negligible portion of the total lipolytic activity of the cheese is contributed by the milk or rennet during making. Of the possible sources of the lipolytic activity, the bacterial flora of the cheese is the most logical. No lipolytic activity is detectable in the green cheese during the first 5–6 days after making, and most of the cheese lipolytic activity is set free between the fifth and hundredth day of the ripening period after making. Since very few of the lactic acid bacteria present in Cheddar cheese are strongly lipolytic in their normal life cycles, it is suggested that the lipolytic activity of cheese at pH 5 may represent endocellular bacterial lipases of these organisms liberated by bacterial autolysis (19).

The lipolytic activity at pH 5 of pasteurized milk cheese also is presented in figure 1. No important differences in the lipolytic activities of raw and pasteurized milk Cheddar cheese during making are apparent. The first important increase in lipolytic activity for pasteurized milk Cheddar cheese during the ripening period occurs between the fifth and twentieth day after making. This increase in lipolytic activity for pasteurized milk cheese occurs considerably earlier in the ripening period than the time at which lipolytic activity is detectable in raw milk cheese. In all pairs of raw and pasteurized milk cheeses examined, the lipolytic activity in the pasteurized milk cheeses was higher on the tenth and twentieth day than in the corresponding raw milk cheeses. It is probable that the factor responsible is related to the differences in flora between raw and pasteurized milk cheese. This early lipolytic activity in pasteurized milk cheese disappears rapidly, as shown in figure 1, and the total lipase content of pasteurized milk cheese at any age after the first 30 days is less than half that of the corresponding raw milk cheese. The bacteria responsible probably are species which are greatly reduced in number during pasteurization.

Since raw milk cheese develops a higher flavor (generally attributed in large part to increased production of volatile fatty acids) than pasteurized milk cheese, it follows that lipases such as those studied in this report are at least in part responsible for more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

SUMMARY

1. The stability and pH activity characteristics of milk lipase are such that this enzyme can play no continuing role in the ripening of Cheddar cheese after making.

2. Analyses made at intervals during the making and ripening of raw milk Cheddar cheese show that milk lipase disappears during the making and is completely absent in the young cheese.

3. The addition of rennet extract during Cheddar cheesemaking causes an increase in lipolytic activity. This increase in lipolytic activity disappears within a period of approximately 30 minutes.

4. After 5 to 20 days, lipases which are considered bacterial begin to make their appearance in the young Cheddar cheese.

5. Most of the lipase active at pH 5 is set free between the fifth and hundredth day of ripening. Lipolytic activity in pasteurized milk Cheddar cheese can be detected in larger amounts between the fifth and twenty-fifth day of the ripening period than in corresponding raw milk Cheddar cheese. At any time after the cheese is 30 days of age, however, the total lipolytic activity of pasteurized milk cheese is less than half that of corresponding raw milk cheese.

6. Bacterial lipases are believed to be at least in part responsible for more rapid flavor development in raw milk Cheddar cheese as compared to Cheddar cheese made from identical milk after pasteurization.

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DETERMINATION OF CHEESE PROTEINASE[1]

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During the past several years one of the major problems to be dealt with in the dairy industry has been concerned with the differences in ripening of raw milk and pasteurized milk Cheddar cheese. In an effort to attack this problem from the enzymatic viewpoint, it was necessary, first, to demonstrate the presence of hydrolytic enzymes (both lipases and proteinases) in the cheese, and, secondly, to devise methods suitable for the quantitative determination of these enzymes. This paper deals only with the proteolytic system of cheese. Lipases in cheese have been dealt with in other publications (2, 3).

The presence of proteolytic enzymes in Cheddar cheese has been generally assumed, and adequate evidence of their presence therein is contained in this report.

DETERMINATION OF CHEESE PROTEINASE

Substrate used and its preparation. Casein was chosen as the substrate to be used in the method as finally adopted, not only because it is reproducible but also because it is very similar to the cheese curd itself.

The substrate is prepared as follows: Ten grams of purified casein (Labco Brand, Casein Company of America) are weighed out with an accuracy of $\pm$ 0.1 g. To the weighed sample in a Waring Blendor or other suitable mixer, 25 ml. of distilled water and 25 ml. of 1 N sodium hydroxide are added. The power mixer is run at low speed for 2 minutes and then at high speed for 5 minutes. At the end of this time, while the mixer is still running, 40 ml. of a 0.2 molar sodium citrate solution are added and the mixer is allowed to run at high speed for 2 minutes longer. The substrate solution then is diluted to an approximate volume of 300 ml. At this point, while the mixer is running at high speed, 2 g. of gum ghatti dissolved in 30–40 ml. of distilled water are added. The total volume of the mixture should be less than 350 ml. The mixer is allowed to run for 5 minutes after the addition of the gum ghatti. A few drops of methyl red now are added. While the mixer is running, concentrated sulfuric acid is added dropwise until a pH of 5 is reached (red to methyl red). After a pH of 5 is reached, the mixer is allowed to run at high speed for 5 more minutes. At the end of this period the substrate preparation is quantitatively re-

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moved from the mixer and diluted to 400 ml. The suspension obtained is run through a small hand homogenizer three to four times. After homogenization, the suspension is filtered through coarse qualitative filter paper to remove extraneous material and then is ready for use. Since substrate adequate for several enzyme analyses is prepared in the preceding operation, enough toluol to saturate the mixture is added as a preservative. Stock substrate solutions should be rehomogenized and filtered each day before use.

Preparation and analysis of cheese samples for proteinase content. Twenty grams of the cheese are weighed out with an accurracy of $\pm$ 0.1 g. To the weighed sample in a Waring Blendor or other suitable mixer, enough distilled water is added to make an approximate volume of 100 ml. The power mixer then is run at low speed for 2 minutes. This length of time is ample to macerate the cheese sample. The mixer then is set at high speed and allowed to run 7 minutes more. At the end of this time the mixture is in the form of a smooth, white cream. This mixture is quantitatively transferred to a 250-ml. graduate with less than 100 ml. of distilled water. The total volume of the mixture then is made to exactly 200 ml. in the graduate. After thorough mixing in the graduate, the suspension is homogenized five to six times through a small hand homogenizer. The cheese sample now is ready to be subjected to enzyme analysis.

In this method for the estimation of proteinase content of Cheddar cheese, a standard suspension of casein is digested under standard conditions, and the undigested casein is precipitated with trichloroacetic acid. The amount of unprecipitated protein split products, which is a measure of the amount of proteinase present, is estimated by measurement of the nitrogen contained therein. The nitrogen content of these unprecipitated protein split products, to be referred to hereafter as non-protein nitrogen, is determined by a colorimetric micromethod (1).

The enzyme analysis as finally adopted proceeds as follows: To 5 ml. of substrate suspension, 1 ml. of 1 molar acetate buffer at pH 5, 1 ml. of freshly prepared 0.015 molar cysteine hydrochloride, and 0.2 ml. toluol are added, and the whole is shaken vigorously for 2 minutes. This suspension and the homogenized cheese sample are placed separately in a water bath held at 40° C. When both have attained this temperature, from 0.5 to 3 ml. (depending upon the activity) of the cheese suspension are added to the substrate suspension, and the total volume is made to 10.2 ml. with distilled water. This digestion mixture is shaken vigorously for 30 seconds and then run quickly two to three times through a small hand homogenizer into a small test tube. After the removal of a 1-ml. aliquot for the determination of initial non-protein nitrogen present, the digestion mixture is incubated in a stoppered tube in the water bath at 40° C. for 5 hours. At the end of this time the digestion mixture is rehomogenized as before, and another 1-ml. aliquot is removed for analysis. The difference in non-protein nitrogen

content of the two aliquots represents casein hydrolysis and is a measure of proteinase present.

The analysis of the 1-ml. aliquots for non-protein nitrogen content is carried out as follows: The 1-ml. aliquot is pipetted immediately upon removal from the digestion mixture into 25 ml. of 0.3 N trichloroacetic acid in a large test tube. The tube is shaken vigorously and the contents filtered through a dry filter paper. An 11-cm. filter paper, such as Whatman no. 2, which does not adsorb protein split products, must be used. A suitable aliquot of the filtrate obtained is analyzed for nitrogen content by the colorimetric micromethod (1). The following alternative procedure for nitrogen analysis of the 1-ml. digestion mixture aliquots is used when the cheese samples are low in proteinase content. The 1-ml. aliquot is pipetted immediately upon removal from the digestion mixture into 4 ml. of 1 N trichloroacetic acid in a small test tube. The tube is shaken vigorously, and the contents are filtered through a dry 3-cm. filter paper of the same type as described above. The filtrate obtained is analyzed as above for nitrogen content. It usually is best to run a zero time analysis for each tube in both the above procedures since the non-protein nitrogen blank varies from time to time.

One cheese proteinase unit is defined as the amount of enzyme which, when diluted to 10.2 ml. and incubated for 5 hours under the above conditions, will liberate 1 μg. of non-protein nitrogen. For instance, if 2 ml. of enzyme sample are used per 10.2 ml. of digestion mixture, and 1 ml. of digestion mixture (1/10.2 of the total) is added to 25 ml. of trichloroacetic acid (total volume of 26 ml.), and the observed increase in non-protein nitrogen is 7.5 μg. per 5 ml. of trichloroacetic acid filtrate (5/26 of the total volume), the number of proteinase units present in the 2-ml. enzyme sample is $7.5 \times 10.2 \times 26 \div 5$ or 397.8 units, or 198.9 proteinase units per ml. of enzyme sample. That the relation between amount of enzyme and increase in soluble nitrogen is linear is demonstrated by the experiments summarized in figure 3 and table 2. In figure 3 the relationship between quantity of cheese proteinase and amount of casein hydrolysis is presented. An arbitrary enzyme preparation was used in this experiment. As may be seen, care should be taken that the amount of enzyme preparation used is such that non-protein nitrogen increases of 2.75 μg. or less per ml. of trichloroacetic acid filtrates are obtained. Above this level the casein hydrolysis obtained is not directly proportional to the quantity of proteinase present.

FACTORS AFFECTING THE ACTIVITY OF CHEESE PROTEINASE

Effect of pH on cheese proteinase activity. In figure 1 the pH activity curve for the hydrolysis of cheese proteinase is given. Determinations of enzyme activity at various pH values were carried out according to the procedure outlined previously in this paper. By means of a composite 0.5 molar

acetate, 0.5 molar phosphate, 0.5 molar borate buffer, pH was controlled throughout the pH range desired. Aliquots of this composite buffer were adjusted with 5 N sulfuric acid or 5 N sodium hydroxide to the pH values shown in figure 1. Proteinase determinations at these pH values were then carried out using in place of 1 ml. of 1 molar acetate buffer at pH 5, 1 ml. of the pH adjusted aliquots of the composite buffer.

It will be seen from the figure that casein is split most rapidly at pH 5. There also is a secondary optimum at pH 7 to 8. Other experiments have shown that this secondary pH optimum seems to vary with the age of the cheese. The primary pH optimum of the proteolytic activity, however, seems to be quite constant throughout the cheese ripening period. The

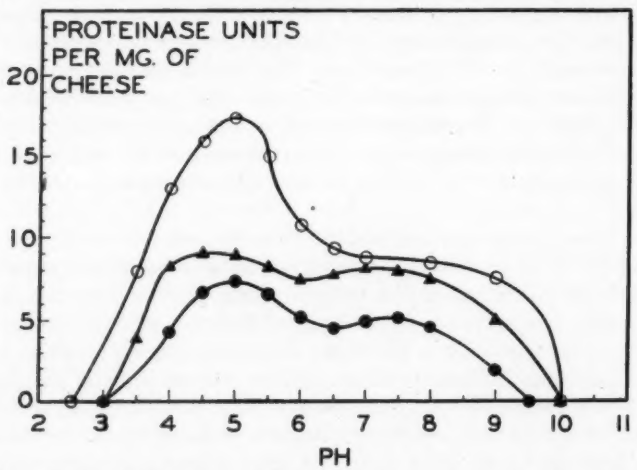

FIG. 1. Effect of pH on rate of casein hydrolysis by cheese extracts. Circles represent enzyme analyses in the presence of 0.015 molar cysteine of extracts of 6-month-old raw milk cheese A–100; triangles represent enzyme analyses without cysteine of extracts of 6-month-old raw milk cheese A–100; and dots represent enzyme analyses in the presence of 0.015 molar cysteine of extracts of 2-month-old raw milk cheese A–100.

presence of two peaks in the pH optimum of cheese proteinase indicates strongly that two or more proteinases are present. This also is indicated by cysteine activation of the enzymes at various pH values (fig. 1). The proteolytic activity at pH 5 is greatly activated by cysteine and probably represents endocellular bacterial proteinases liberated by bacterial autolysis (4). The proteolytic activity at pH 7 and above is unaffected by cysteine and probably represents extracellular proteinases liberated by bacteria during their normal life cycle. Since the pH of ripening cheese remains at or very near 5, only the proteolytic activity at pH 5 present in the cheese is important.

TABLE 1

The effects of activators and buffers on cheese proteinase activity

Substance	Concentration	Apparent cheese proteinase in sample
	M	*(units/mg. cheese)*
	Activators	
None	.....	25.9
Potassium cyanide	0.01	64.5
Sodium sulfide	0.01	35.2
Cysteine hydrochloride	0.01	70.8
	Buffers*	
Aniline	0.04	29.2†
Pyridine	0.1	21.4
Phosphate	0.1	29.2
Borate	0.1	29.3
Phthalate	0.1	27.3
Acetate	0.1	29.4

* All buffers were adjusted to pH 5 with 5 N sulfuric acid or 5 N sodium hydroxide prior to proteinase determination.

† The proteinase solution used in the buffer experiments was not the same as that used in the activator experiments.

Effect of buffers on cheese proteinase activity. In the development of a method for the estimation of cheese proteinase, it was necessary to find a buffer which did not affect the proteinase activity and which had good buffering capacity in the desired pH range. In table 1 are shown the effects of six buffers upon the activity of cheese proteinase. The acetate buffer appears to meet the requirements and also is convenient.

Effect of activators on cheese proteinase activity. Various reducing agents known to possess definite activating properties for proteinases such as papain were tested for activating effect on cheese proteinase. As may

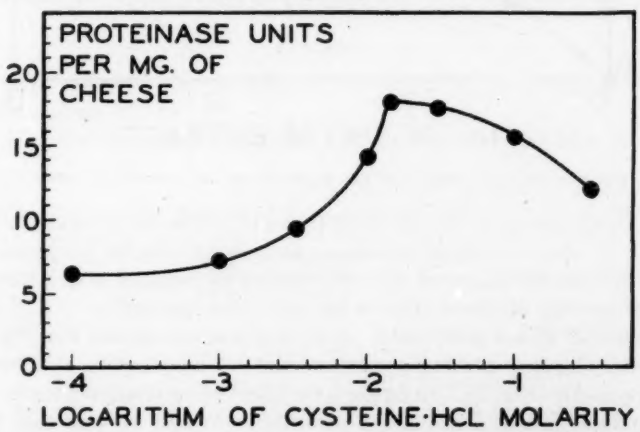

FIG. 2. Effect of cysteine hydrochloride concentration on cheese proteinase activity.

be seen from table 1, activation of the enzyme was obtained in all cases. Cysteine, however, gave more complete activation than sodium sulfide or potassium cyanide.

In order to determine the concentration of cysteine necessary to give maximum activation of cheese proteinase at pH 5, an experiment was run determining the proteinase activity of an enzyme preparation at various levels of cysteine hydrochloride. The results are given in figure 2. It may be seen that maximum activation of cheese proteinase occurred when the

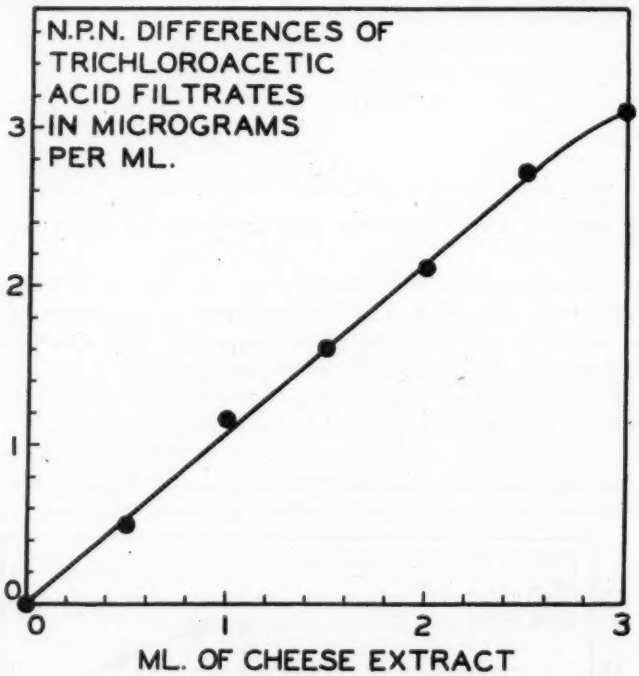

FIG. 3. Relation between quantity of cheese proteinase and amount of casein hydrolysis.

cysteine was present at a level of 0.015 molar. The use of cysteine hydrochloride in order to insure maximum activity of enzyme preparations at pH 5 then was incorporated into the method as outlined under "Preparation and analysis of cheese samples for proteinase content".

Kinetics of cheese proteinase. It already has been shown that the casein hydrolysis obtained is directly proportional to the quantity of cheese proteinase present (Fig. 3). In figure 4 the effect of incubation time on casein hydrolysis by cheese proteinase is presented. It may be seen that the hydrolysis of casein by cheese proteinase at pH 5 is linear up to the time when

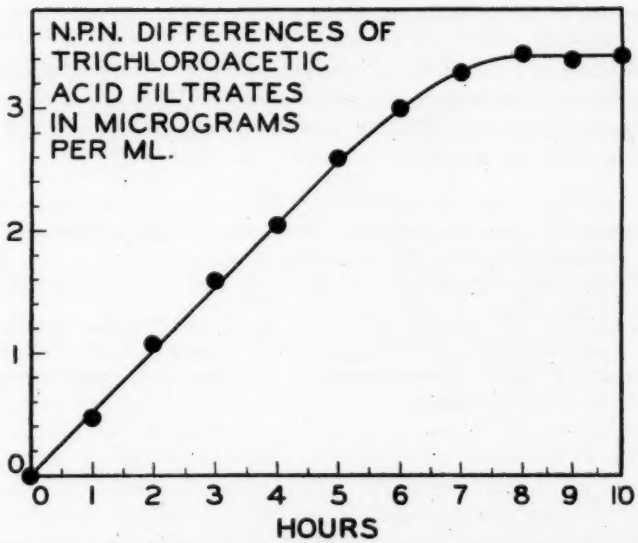

FIG. 4. Effect of incubation time on casein hydrolysis by cheese proteinase.

the increase of protein split products in the trichloroacetic acid filtrate is equivalent to 2.60 μg. non-protein nitrogen per ml.

Reliability of the method. The reliability of the method was checked by running varying concentrations of the same enzyme preparation. The results may be found in table 2. As may be seen, determinations run at various sample levels agree within 10 per cent.

SUMMARY

1. Data are presented showing the presence of proteolytic activity in Cheddar cheese.

2. A method is presented for the quantitative determination of proteo-

TABLE 2

Effect of sample size on apparent cheese proteinase content of a cheese extract

Sample size	Non-protein nitrogen increases in trichloroacetic acid filtrates	Proteinase content
(*ml.*)	(μg./*ml.*)	(*units/ml.*)
1.0	0.72	191
1.0	0.79	210
2.0	1.57	208
2.0	1.52	202
3.0	2.36	209
3.0	2.30	203

lytic activity in cheese. Determinations run at various sample levels agree within 10 per cent.

3. Hydrolysis of casein by the Cheddar cheese proteinase system is most rapid at pH 5. A secondary optimum occurs at pH 7 to 8.

4. Proteolytic activity of cheese suspensions at pH 5 is enhanced in the presence of reducing agents.

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PROTEINASE CONTENT OF CHEDDAR CHEESE DURING MAKING AND RIPENING[1]

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It is a well recognized fact that proteinases play an important part in the ripening of Cheddar cheese because of their action on the cheese curd. Possible sources of cheese proteinases are the proteinases of milk, of rennet, and of microorganisms. It was first suggested by Babcock and Russell (1) and Babcock *et al.* (5) that an inherent milk proteinase, termed galactase, probably is the most important proteolytic agent in cheese ripening. Later work on variation of rennet levels in cheesemaking caused Babcock *et al.* (3) to revise their early views. Their final conclusion was that rennet is the most important proteinase source in cheese, its protein digestive action being due to the action of pepsin present in the rennet as an impurity. Sherwood (12), however, reported that the use of pepsin for rennet in Cheddar cheese resulted in 40–50 per cent less protein degradation than took place in normal rennet control cheeses. The ratios between amounts of different protein split products were quite similar for both types of cheese, indicating that nitrogen partition was identical. Sherwood (11) also found that although the extent of protein degradation in chloroform-treated Cheddar cheese was considerably less than for normal Cheddar cheese controls, the general course of nitrogen partition was the same for both chloroform-treated and normal cheese. The same work showed that normal Cheddar cheese had consistently higher levels of subpeptone nitrogen than chloroform-treated cheese. From these data Sherwood concluded that rennet itself is the most important proteolytic agent in Cheddar cheese ripening, and since its action extends but little beyond the peptone stage, any further proteolytic degradation must be due to bacterial action.

The purpose of the present investigation is to establish the relative importance of various sources of cheese proteinases, and also to present studies of the differences in proteolytic activity during the making and ripening of raw and pasteurized Cheddar cheese made from the same milk.

METHODS

Cheese proteinase. The method used to determine proteolytic activity in Cheddar cheese reported by the present authors (10) was used in all cheese proteinase analyses. All analyses were made both with and without 0.015

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molar cysteine in order to differentiate between cysteine-activated proteinases and those unaffected by cysteine.

Cheesemaking procedure. The milk used in making the experimental cheeses was mixed, raw, whole milk from the University Dairy. A 920-lb. lot of the milk was divided into two equal portions. The first portion received no treatment. The second portion was subjected to pasteurization (holder method—30 min. at 145° F.). Each portion, after treatment, was placed in a separate vat, and both received identical subsequent treatment. Cheeses were made by an exprienced maker according to standard procedures. From each vat two 22-lb. cheeses were obtained. The green cheeses produced were kept at 68° F. for a period of 2–3 days and then were transferred to a cold room (40–45° F.) for the remainder of the ripening period.

Sampling. Samples for enzyme analysis were taken at various intervals during the making process and ripening period. Sampling times used are indicated in the figures and tables.

PROTEOLYTIC ACTIVITY OF CHEDDAR CHEESE DURING MAKING AND RIPENING

Proteinase content of raw and pasteurized Cheddar cheese. In figures 1–*a*, 1–*b*, 2–*a*, and 2–*b* the averages of the proteolytic activities at pH 5 of a series of pairs of pasteurized milk cheeses and raw milk cheeses made by the procedure described under "Methods" are given. Figures 1–*b* and 2–*b* cover the entire ripening period. Figures 1–*a* and 2–*a* show, on an enlarged scale, the changes occurring during the making and the first few days of ripening.

Figures 1–*a* and 1–*b*, representing the proteolytic activity in raw milk Cheddar cheese, will be considered first. In figure 1–*a*, it will be noted that a small amount of proteolytic activity is present in the milk at the time it is placed in the vat. This represents milk proteinase, the significance of which will be discussed later. The first important increase in proteolytic activity occurs 90 to 120 minutes after the start of making. While part of this increase can be attributed to proteinases of the added rennet, comparison of figures 1–*a* and 2–*a* indicates that most of this increase probably is due to bacteria growing and producing extracellular proteinases during the making process. Since the corresponding increase in the proteolytic activity of pasteurized milk cheese (fig. 2–*a*) is much smaller, it is believed that the bacteria responsible are destroyed in the pasteurization process. No further change in proteolytic activity occurs until after the cheese is in its final form and has been stored. In the first few days of ripening a large increase in cysteine-activated proteinases occurs. The use of cysteine here is valuable in that it affords a means of differentiation in the type of proteinases present in the cheese and thus affords a clue to the source of these

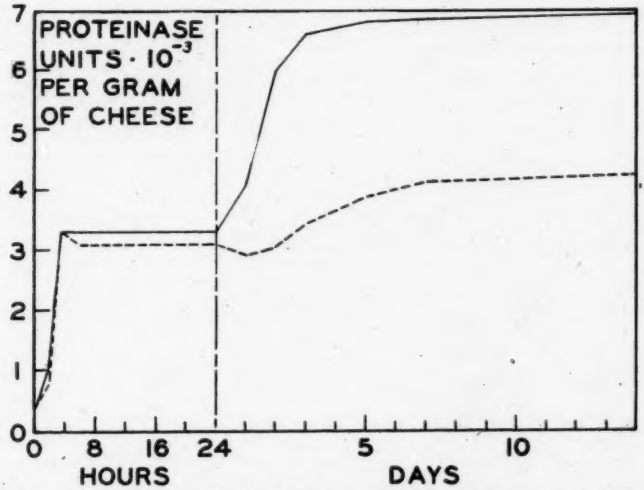

FIG. 1–*a*. Proteinase activity at pH 5 in raw milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

proteinases. It should be emphasized that in the ripening cheese where strictly anaerobic conditions are present (6), the cysteine-activated proteinases undoubtedly are present in a fully active state.

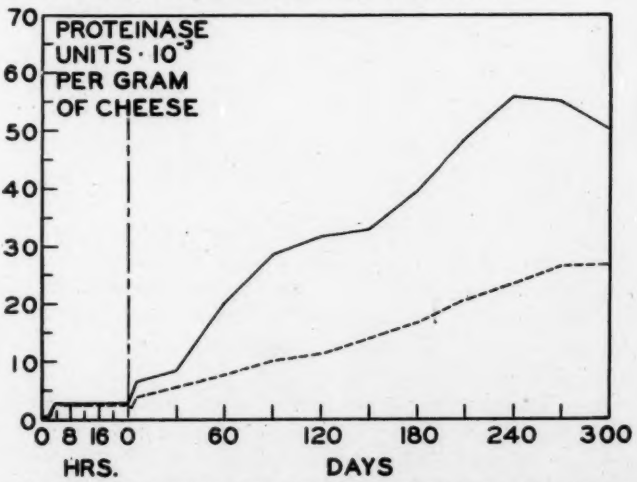

FIG. 1–*b*. Proteinase activity at pH 5 in raw milk Cheddar cheese during making and a 300-day ripening period, same cheeses as Fig. 1–*a*. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine; broken line, without cysteine.

Figures 1–*a* and 1–*b* show that the amount of proteinase in the milk is very small compared to the proteinase of the cheese. Consequently, even if the milk proteinase is carried over quantitatively into the cheese, its role in ripening must be small. It also will be noted that the proteolytic activity at pH 5 of the added rennet is relatively small; it is readily seen, therefore, why such large amounts of rennet extract must be added to Cheddar cheese in order to increase markedly the rate of ripening (2, 3, 4, 7, 8, 9, 14).

Figure 1–*a* indicates that most of the proteinase of the young cheese is bacterial in origin. Part of this is produced in the vat, and the remainder is set free during the first few days of ripening. Figure 1–*b* shows that as

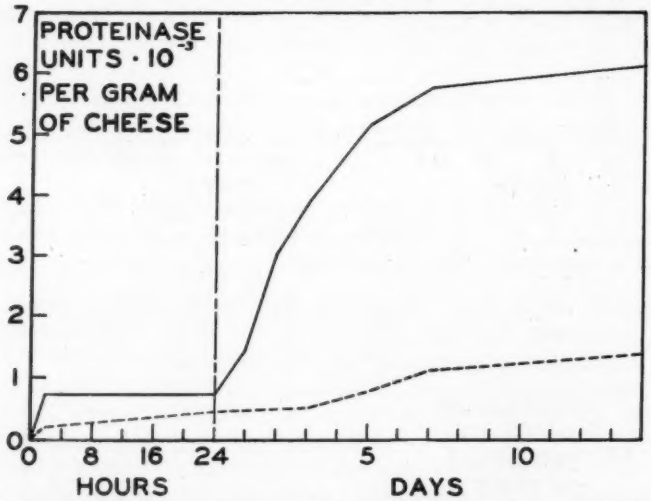

FIG. 2–*a*. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

the cheese ripens its proteinase content increases greatly. It is very apparent that only a small portion of the total proteolytic activity is contributed by the milk and the rennet when standard Cheddar cheesemaking procedures are employed. The greater portion of the proteinase increase is cysteine-activated and may represent endocellular bacterial proteinases liberated by bacterial autolysis (13).

The proteinase activity of pasteurized milk cheese at various ages is shown in figures 2–*a* and 2–*b*. In pasteurized milk cheese (fig. 2–*a*) there is no increase in proteinase in the vat other than that introduced with the rennet. There is a relatively large increase in cysteine-activated proteinase during the first few days of ripening. Figure 2–*b* shows that the total proteinase content of pasteurized milk cheese during ripening is less than that

of raw milk cheese, and that in pasteurized milk cheese much less cysteine-activated proteinase is present.

Since the sources of the proteinases found in Cheddar cheese during the later ripening period must be in large part bacterial, it might readily be assumed that some of the differences between raw and pasteurized milk cheese made from the same lot of milk could be attributed to this difference in cysteine-activated proteinase content. The bacteria responsible probably are species which are greatly reduced in number during pasteurization. It also may be seen from figures 1–*b* and 2–*b* that the content of proteinase, not activated by cysteine, becomes greater in the pasteurized milk cheese than

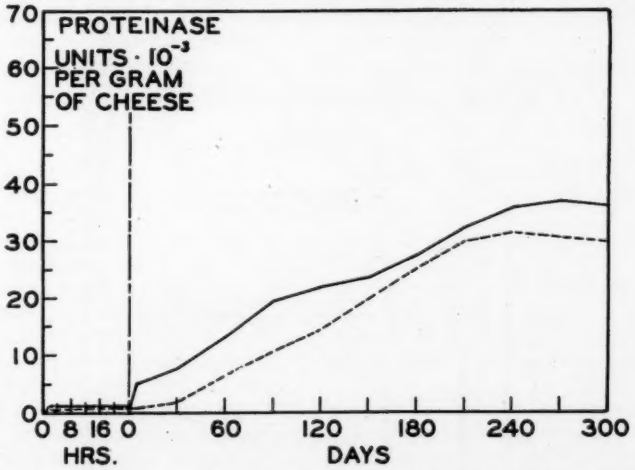

FIG. 2–*b*. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and a 300-day ripening period, same cheeses as Fig. 2–*a*. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine: broken line, without cysteine.

that of the raw milk cheese after 80 days of ripening and remains greater throughout the rest of the ripening period.

During ripening, flavor development always is more complete in raw milk cheese than in pasteurized milk cheese of the same age. Since the difference in proteinase content between the two types is largely in the cysteine-activated fraction, it follows that cysteine-activated proteinases may be responsible, in part, for the more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

Reproducibility of cheeses. The data of figures 1 and 2 represent aver-

TABLE 1

Proteinase content of Cheddar cheese during ripening

Cheese no.*	Proteinase content in units per milligram of cheese									
	With cysteine†					Without cysteine				
	0 days	10 days	30 days	90 days	180 days	0 days	10 days	30 days	90 days	180 days
1015R	3.5	6.6	8.2	27.5	36.6	2.6	4.1	5.1	9.5	16.3
1022R	3.0	6.2	7.5	21.3	33.1	2.1	3.8	4.8	9.2	15.8
1103R	3.9	7.0	8.3	30.1	42.7	2.5	4.3	5.0	9.8	17.0
1119R	4.1	7.0	8.4	31.2	41.7	2.8	4.5	5.3	10.0	17.4
1124R	3.6	6.7	8.1	29.0	41.3	2.6	3.9	5.1	9.6	16.5
1201R	3.5	6.4	8.0	28.0	34.8	2.4	3.7	5.0	9.6	15.2
1015P	0	5.5	7.2	18.0	25.8	0	1.1	1.9	9.3	27.2
1022P	0	4.9	7.7	17.5	22.1	0	0.8	1.4	10.0	26.5
1103P	0	5.7	7.6	18.9	29.1	0	1.2	1.5	10.1	28.1
1119P	0	6.0	7.9	17.4	28.5	0	1.5	2.1	10.4	30.1
1124P	0	5.6	7.0	17.7	24.9	0	1.0	1.6	8.9	26.2
1201P	0	5.4	7.2	19.9	22.9	0	1.0	1.0	8.7	24.7

* The letter R indicates a raw milk cheese, P a pasteurized milk cheese. Cheeses made from the same lot of milk have the same number.
† Proteinase determinations were made at pH 5 both with and without 0.015 M cysteine (10).

ages of six pairs of raw and pasteurized milk cheeses. Analyses after 180 days, however, were made on only one pair of cheeses. The individual curves for the six pairs of cheeses were very similar. The individual analyses are presented in table 1 and may be seen to check closely.

SUMMARY

1. The proteinase content of a series of pairs of raw and pasteurized milk Cheddar cheeses has been determined at intervals during the making and ripening periods.

2. The active proteinase in ripening Cheddar cheese is largely of bacterial origin; only a small fraction of the total activity is contributed by the milk and the rennet.

3. Pasteurized milk Cheddar cheese is characterized by a lower content of cysteine-activated proteinase than raw milk Cheddar cheese.

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THE HERITABILITY OF OFFICIAL TYPE RATINGS AND THE CORRELATION BETWEEN TYPE RATINGS AND BUTTERFAT PRODUCTION OF AYRSHIRE COWS[1]

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The selection of dairy cattle for breeding purposes usually is based on the type and the production records of the animals, their ancestors or their progeny. The consideration given to type in a selection program should be determined by its economic importance to the breeder—*i.e.*, by the advertising value and the increase in selling prices of animals with high classification ratings, by the relationship between type and production, and by the heritability of type. The economic importance of type varies from herd to herd, depending on the sales policy used by the breeder. Neither the correlation between type and production nor the heritability of type should vary much from herd to herd within a breed.

The purpose of this study was to estimate the heritability of single type ratings and to compute the correlation between type and production in Ayrshire cattle.

EXPERIMENTAL PROCEDURE

The data used in this study were the type ratings of Ayrshire cows classified between March, 1942, and May, 1946, by official inspectors in accordance with the rules of the classification program of the Ayrshire Breeders' Association. The participation of Ayrshire herds in this program is voluntary. According to the plan, the breeder submits for official inspection all of his cows that have freshened one or more times. These cows are given a single rating of one of five grades: Excellent, Very Good, Good Plus, Good, and Fair.

The rating and other information on each cow were coded and punched on International Business Machine cards. These cards were sorted according to inspector and date of classification. The correlation coefficients between ratings of related animals that were classified on the same day by the same inspector were computed and used to estimate the proportion of the total variability in ratings that is transmitted from parents to their offspring. In this paper this transmitted portion is called the heritability. From the correlation between the type ratings of paternal sisters and the regression of daughter's rating on dam's rating after removing the influence of the sires, the heritability of single type ratings was estimated.

The butterfat production records of classified cows were used to calculate

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the correlation between type and production. Type was paired with the first record, with the record begun nearest to the date of classification, and with the average of all records. All butterfat records were adjusted to a mature-equivalent, 305-day, twice-a-day milking basis.

RESULTS

Heritability of type. The classification ratings of 3,738 cows sired by 368 bulls were used to compute the correlation between the ratings of paternal sisters. Only those sires with six or more daughters that were classified the same day were included in this study. This procedure should have eliminated most of those sires which would be represented by a few highly selected mature daughters. The frequency distribution of the ratings of these 3,738 cows and the distribution of 8,573 Ayrshire cows that were classified during the same period are given in table 1. The agreement between the two columns showing percentages indicates that the group of 3,738 cows is a representative sample of the breed.

TABLE 1

Frequency distribution of the ratings for paternal sisters and for the Ayrshire breed

Rating	No. of paternal sisters	Percentage of total	Breed percentage of total*
Excellent	109	2.9	3.7
Very good	831	22.2	22.1
Good plus	1627	43.6	42.7
Good	987	26.4	26.8
Fair	184	4.9	4.7
Total	3738	100.0	100.0

* This is the distribution of the classification ratings of 8,573 Ayrshire females classified between March, 1942, and May, 1946.

The variance of these type ratings was divided into variation between inspectors, between sires within inspectors, and between daughters by the same sire, as shown in table 2 (9). The standard deviation of all ratings was 0.9. The variance component A represents variation between daughters by the same sire, while component B is the additional variance which can be ascribed to difference between sires, and C to differences between inspectors. The ratio $\frac{B}{A+B}$ is the average correlation between daughters by the same sire. In these data this correlation was 0.12 with a standard error of 0.04.

There were 1,601 cows whose dams were classified on the same day by the same inspector. These cows were sired by 789 sires. The analysis of covariance technique was used (table 3) to obtain the regression of daughter's rating on dam's rating within sires and this coefficient was 0.14 ± 0.034. The regression of offspring on dam within sires avoids much

TABLE 2

Analysis of the variation in type ratings of 3,738 cows sired by 368 bulls

Source of variation	Degrees of freedom	Mean square	Variance components of mean square*	Individual components
Between inspectors	9	15.330†	$A + 10.16\ B + 373.7\ C$	$C = 0.037$
Between sires within insps.	358	1.634†	$A + 10.16\ B$	$B = 0.095$
Within sires within insps.	3370	0.667	A	$A = 0.667$
Summary	3737	0.795	$A + 0.998\ B + 0.9\ C$ $\frac{B}{A+B} = 0.12$	$A + B + C = 0.799$

* Coefficients for the components of variance were computed by the method used by Dickerson (4).
† $P < 0.01$.

of the environmental contribution which would be found in a gross correlation or regression between parent and offspring.

Estimates of the heritability[2] of type in cattle can be made from the above relationship. In a random breeding population the correlation between traits of paternal sisters may be expected to contain 0.25 of the additive genetic variance, 0.06 or less of the epistatic variance, plus the environmental portion of the variance times the correlation between the environments of the half sisters (10). However, in those herds where the only source of new stock is through sires, the average genetic relationship between two animals by the same bull often is greater than 0.25. In most cases the animals used in this study were from herds using only one or two sires. Therefore, the average genetic relationship of the paternal sisters should be approximately 0.30. Since the environmental contribution to the

TABLE 3

Analysis of covariance of type ratings of dams and daughters and the intra-sire regression of daughter's rating on dam's rating

Source of variation	Degrees of freedom	Sums of squares		Cross products	Regression dau. on dam
		Daughters	Dams		
Between sires	788	719	830	240	.....
Within sires	812	452	481	66	0.14
Total	1600	1171	1311	306	.....

paternal sister correlation could not be determined accurately, the heritability of type ratings could be estimated to be somewhat less than $\frac{1}{0.30} \times 0.12$ or 0.40 from these data.

The intra-sire regression coefficient of daughter's rating on dam's rating should contain 0.5 of the additive genetic variance plus about 0.25 of the epistatic variance. Non-random matings would not affect the intra-sire regression coefficient of daughter on dam (6). Hence, the estimate of heritability from these data would be about 2×0.14 or 0.28.

When the two estimates of heritability are combined, the average figure is 0.30 and the 95 per cent fiducial limits are 0.19 and 0.42. This means that the chances are very good (19 out of 20) that the real heritability of type ratings lies within these limits.

Correlation between type and production. The official ratings, number of cows, percentage of total, and average butterfat production (first record, record started nearest to the date of classification, and the average of all records) of the 5,177 cows that were classified and also had production

[2] Lush defined the degree of heritability as "the fraction of the observed variance which was caused by differences in heredity" (6). This fraction may result from additive gene effects, interaction of allelic genes (dominance), and interaction of non-allelic genes (epistasis).

records are given in table 4. On the basis of the percentages for the classification groups for all classified Ayrshires (table 1), a larger percentage of the Excellent and Very Good cows have production records. This is similar to Copeland's findings (1, 2) in his study of classified Jersey cows, and means either that the herds on official test were culled more closely before the inspector classified the cattle, that these herds were inherently better in type conformation than the herds that were not on test, or that inspectors are influenced by the records (consciously or unconsciously) and thereby place higher producers in the Excellent and Very Good classes when they know about production and do not when the production is unknown to them.

The butterfat production of cows increased with each rise in type rating with the exception of the average of the first butterfat records of the Good and Fair groups of cows.

In order to remove the effect of herd on the correlation between type and

TABLE 4

Frequency distribution of the ratings of the cows with production records and the average production of butterfat for each rating

Classification rating	No. of cows	Percentage of total	Av. butterfat production		
			1st records	Nearest record	Av. all records
Excellent	320	6.2	415	417	412
Very good	1479	28.6	394	385	386
Good plus	2105	40.6	376	367	369
Good	1097	21.2	361	352	353
Fair	176	3.4	363	345	350
Summary	5177	100.0	380	371	372

butterfat production, the data were analyzed by the analysis of covariance to obtain the intra-herd correlation and regression of butterfat production on type rating. The correlation and regression coefficients of the first, nearest, and average of all butterfat records on type rating are given in table 5. The between-herd correlation coefficients, $r = 0.53$, 0.42, and 0.50, are highly significant and mean that those herds classified as the best type tended to have the highest average production per cow. Within each herd, however, the correlation coefficients between type and butterfat production were lower, $r = 0.16$, 0.16, and 0.19, but still statistically significant. The regression coefficients show that within a herd the butterfat production of the cows increased about 12–14 lb. for each increase in type grade. However, herds that averaged one classification grade higher in type also averaged about 82 lb. more butterfat per cow.

DISCUSSION

The objectives of this study in dairy cattle breeding were to determine the heritability (transmitted portion of the superiority of parents over

average of group from which parents were selected) of type and the correlation between type and production. On the basis of the studies of economic characteristics in swine (3), and milk and butterfat production in dairy cattle (8), it would seem that the results found here might well apply to other breeds of dairy cattle.

The results have shown that the heritability of single type ratings in Ayrshire cows probably lies between 0.19 and 0.42. This indicates that selection for type through a type classification program should improve the type of future generations of dairy cattle. If type was considered in the selection program, the breeder could expect the next group of offspring from the selected parents to be approximately the heritability times the difference between the type of the selected parents and the type of the group or herd from which the parents came. For example, if the average type of a herd was Good Plus (82.5) and the animals selected as parents averaged Very

TABLE 5

Analysis of covariance and the correlation and regression coefficients of butterfat production on type rating

Source of variation	Degrees of freedom	Correlation coefficients*			Regression coefficients*		
		1st record & type	Nearest record & type	Av. all records & type	1st record on type	Nearest record on type	Av. all records on type
Between herds	303	0.527	0.417	0.497	82.6	80.3	83.2
Within herds	4873	0.156	0.159	0.193	11.6	13.9	12.9
Summary	5176	0.193	0.184	0.220	15.7	17.7	16.8

* $P < 0.01$ for all values found.

Good (87.5), then the best estimate of the type of the offspring of these parents is $82.5 + 0.2(87.5 - 82.5)$, or 83.5 if the heritability of type is 0.2, and 84 and 84.5 if the heritability is either 0.3 or 0.4, respectively. Actually, the improvement in type would not be this fast unless the sire's breeding value for type was as equally well known as the type of the cows selected as parents. Estimates of a sire's breeding value for type could be made from a progeny test or from a pedigree study. The correlation between the type of a sire and the type of his daughters is not well known, although Copeland's figures indicated a low relationship (1).

The intra-herd relationship of type and butterfat production was 0.16 between type and single records, and 0.19 between type and average of all records. These correlation coefficients are very similar to those reported by Lush (7) for Holstein-Friesian cows, but somewhat smaller than the coefficients which Copeland (2) computed between type and butterfat records of Jersey cows. The difference between the correlation coefficient between type and a single butterfat record (first or nearest) and the correlation

coefficient between type and the average of all butterfat records is not statistically significant, but it is in the expected direction.[3] Intra-herd correlations were computed for this study because in most cases selection is practiced among animals that are raised on the same farm. Selection within herds for type only would not result in any substantial increase in butterfat production. Likewise, selection for butterfat alone would not increase the type of the animals to any great extent.

Hazel (5) has described a procedure to derive selection indexes. Before an index can be made for butterfat and type, however, the relative economic importance of each must be determined, and likewise the correlation between the genotypes (breeding value) for type and butterfat must be known. The advantage of an index is that improvement would be made in both type and production so that the gain would mean most to the breeder in dollars and cents. It seems probable that other characteristics (such as longevity and reproductive behavior) should be considered in deriving an index for selecting dairy cattle.

SUMMARY

The classification ratings of 3,738 paternal sisters and of 1,601 cows out of classified dams were used to estimate the portion of the differences in official type ratings that is transmitted from parents to offspring. This portion is called heritability and from these data this figure is estimated to be 0.3. Thus offspring inherit about one-third of the observed superiority of the parents' type, and herd improvement in the type of dairy cattle can be made by the selection of animals that are above the herd average in type for parents of the next generation.

The correlation between classification rating and production of butterfat was computed from data on 5,177 cows. Within herds there was an average increase of 13 lb. of butterfat for each increase of one grade of type. The relationships between the classification rating and the first butterfat record of a cow, between the type rating of a cow and her butterfat record begun nearest the date of classification, and between the type rating of a cow and the average of all her butterfat records were statistically highly significant, but they were so low that their practical significance is quite small. The correlation coefficients were 0.16, 0.16, and 0.19, respectively.

ACKNOWLEDGMENTS

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[3] The average number of records per cow was 3.5. If the repeatability of butterfat records is assumed to be 0.4, then the expected correlation between one type rating and the average of all butterfat records is 0.16×1.32 or 0.21.

Economics Department, West Virginia University, and Miss Margaret Cross for the sorting and tabulation of the International Business Machine cards. They also are indebted to Dr. H. O. Henderson, Head, Department of Dairy Husbandry, West Virginia University, and Dr. A. B. Chapman, Department of Genetics, University of Wisconsin, for their valuable suggestions in the preparation of this manuscript.

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VARIATIONS IN TYPE RATINGS OF INDIVIDUAL AYRSHIRE COWS[1]

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Type classification programs have been adopted by all the major dairy cattle breed associations. With the exception of the Guernsey breed, in order to participate in this program each breeder must submit all of the registered cows in his herd for classification. At a subsequent reclassification of the herd new ratings on previously classified cows will be official only if the new rating is higher than the previous official rating. Before a definite appraisal of the type classification program can be made, it is desirable to know as much as possible about the amount of variation that occurs when the same cows are classified several times throughout a lifetime. This investigation sought to determine: (a) The degree or amount of variation that occurs when the same cows are classified several times in their lifetime, (b) the degree of agreement between ratings given the same cow by the same inspector and by different inspectors at different times, (c) the possibility that age of animal, stage of lactation, and condition of animal influence the official type rating, (d) the value of photographic records in studying the type of animals from birth to maturity, and (e) the reasons why the ratings of some cows undergo large changes.

EXPERIMENTAL PROCEDURE

The data for this investigation were collected from the Ayrshire herd (known as the Reymann Memorial Herd) at the West Virginia Agricultural Experiment Station. This herd represents approximately 25 years of a continuous, carefully controlled breeding project. No females have been brought into the herd in that period of time, and each normal female that is dropped in the herd must be raised and retained through at least one 305-day lactation before her disposal. The feeding and management of the herd has been kept as nearly constant as possible over the 25-year period. Each animal is bred to freshen each year, allowing 6 to 8 weeks for the dry period.

From 1942 through 1946 all the females that had calved were rated for type approximately three times yearly. During this period 138 cows were rated. Of this number, 32 were rated once, 26 twice, 17 three times, 8 four times, 6 five times, 9 six times, 12 seven times, 9 eight times, 5 nine times, 4 ten times, 2 eleven times, 3 twelve times, and 5 thirteen times. In

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TABLE 1

Classification ratings of cows in the Reymann Memorial Herd rated three times or more from May 16, 1942, to December 20, 1946

Cow no.	Inspector and date													
	R 5–42	R 10–42	S 1–43	R 5–43	T 7–43	U 10–43	U 1–44	V 4–44	W 12–44	X 4–45	Y 12–45	T 5–46	Z 12–46	Range
462	3*	3	3	4	3	3	3	.....	.....	.....	.....	.....	.....	1
473	5	5	5	5	5	5	5	5	.....	.....	.....	.....	.....	0
491	4	4	3	4	4	4	4	3	.....	.....	.....	.....	.....	1
500	4	5	5	5	5	5	5	5	4	4	.....	.....	.....	1
501	4	4	4	5	5	5	5	3	4	4	4	5	4	2
521	4	3	2	3	2	1	.....	.....	.....	.....	.....	.....	.....	3
526	3	3	3	4	3	4	4	4	4	3	5	4	4	2
530	4	4	4	4	3	4	4	4	3	.....	.....	.....	.....	1
537	5	4	4	5	4	4	4	.....	.....	.....	.....	.....	.....	1
539	4	4	4	4	4	4	4	3	3	4	4	4	4	1
561	4	4	4	4	4	4	4	4	5	5	5	.....	.....	1
569	2	4	4	4	2	4	3	.....	.....	.....	.....	.....	.....	2
577	4	4	4	4	4	4	4	3	4	4	.....	.....	.....	1
592	3	4	4	4	4	4	4	3	4	.....	.....	.....	.....	1
611	3	3	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
612	3	3	2	2	3	3	3	2	3	.....	.....	.....	.....	1
614	3	3	4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
616	3	3	3	4	3	4	3	3	3	3	4	4	4	1
617	4	3	4	4	4	4	4	4	.....	.....	.....	.....	.....	1
620	3	4	4	4	3	4	4	3	3	3	3	2	.....	2
622	2	2	3	2	3	3	3	4	2	4	2	4	3	2
624	2	2	3	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
627	2	2	4	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	2
638	2	3	3	2	4	3	3	.....	.....	.....	.....	.....	.....	2
640	3	2	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
641	3	3	3	3	2	3	2	4	.....	.....	.....	.....	.....	2
642	3	3	3	3	3	3	.....	.....	.....	.....	.....	.....	.....	0
644	2	3	2	2	2	3	3	4	.....	.....	.....	.....	.....	2
657	2	2	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
660	.....	3	4	4	4	4	4	5	4	4	3	5	4	2
661	.....	2	2	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
662	2	2	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
664	.....	2	2	2	3	3	3	.....	.....	.....	.....	.....	.....	1
665	.....	2	2	3	2	3	3	2	3	3	.....	.....	.....	1
666	.....	3	3	3	3	3	3	.....	.....	.....	.....	.....	.....	1
668	.....	3	3	2	2	3	4	4	.....	.....	.....	.....	.....	0
669	.....	.....	3	2	2	2	2	1	.....	.....	.....	.....	.....	2
671	.....	2	3	2	3	3	1	3	3	3	.....	.....	.....	2
672	.....	3	3	2	2	3	.....	.....	.....	.....	.....	.....	.....	1
673	.....	3	2	1	2	.....	.....	.....	.....	.....	.....	.....	.....	2

TABLE 1 (*Continued*)

Cow no.	Inspector and date													
	R 5–42	R 10–42	S 1–43	R 5–43	T 7–43	U 10–43	U 1–44	V 4–44	W 12–44	X 4–45	Y 12–45	T 5–46	Z 12–46	Range
674	.....	4	3	3	3	3	3	4	.....	.....	.....	.....	.....	1
675	.....	3	3	2	2	3	2	2	.....	.....	.....	.....	.....	1
676	.....	4	3	3	2	4	3	3	.....	.....	.....	.....	.....	2
677	.....	2	2	2	2	2	.....	.....	.....	.....	.....	.....	.....	0
678	.....	2	2	3	2	3	3	2	3	4	2	3	4	2
679	.....	1	1	1	1	2	2	1	2	1	1	.....	.....	1
680	.....	3	2	3	2	3	3	2	2	1	1	.....	.....	2
681	.....	2	3	2	2	3	2	1	4	.....	.....	.....	.....	3
683	.....	.....	4	4	4	4	4	2	5	3	4	5	5	3
684	.....	.....	3	3	3	3	3	2	.....	.....	.....	.....	.....	1
685	.....	.....	.....	.....	.....	2	2	2	1	.....	.....	.....	.....	1
686	.....	.....	.....	.....	.....	.....	3	3	4	4	4	4	.....	1
687	.....	.....	.....	.....	.....	.....	3	4	4	3	4	4	3	1
688	.....	.....	.....	.....	.....	4	4	4	4	4	5	5	5	1
689	.....	.....	.....	.....	.....	4	4	2	3	.....	.....	.....	.....	2
690	.....	.....	.....	.....	.....	.....	4	4	5	3	4	4	5	2
691	.....	.....	.....	.....	.....	.....	.....	3	4	3	3	3	.....	1
692	.....	.....	.....	.....	.....	.....	.....	4	4	4	4	5	4	1
693	.....	.....	.....	.....	.....	3	3	2	.....	.....	.....	.....	.....	1
695	.....	.....	.....	.....	.....	.....	2	2	2	2	3	.....	.....	1
698	.....	.....	.....	.....	.....	4	4	4	3	3	3	5	5	2
699	.....	.....	.....	.....	.....	.....	3	4	4	3	4	4	4	1
700	.....	.....	.....	.....	.....	.....	.....	.....	3	3	2	3	2	1
701	.....	.....	.....	.....	.....	.....	3	3	2	.....	.....	.....	.....	1
702	.....	.....	.....	.....	.....	.....	2	2	2	3	3	3	2	1
703	.....	.....	.....	.....	.....	.....	3	3	3	3	3	4	4	1
704	.....	.....	.....	.....	.....	.....	.....	3	4	2	3	4	3	2
705	.....	.....	.....	.....	.....	.....	.....	2	2	2	.....	.....	.....	0
707	.....	.....	.....	.....	.....	.....	.....	.....	4	2	2	2	2	2
714	.....	.....	.....	.....	.....	.....	.....	.....	3	3	4	3	.....	1
715	.....	.....	.....	.....	.....	.....	.....	.....	4	3	3	.....	.....	1
716	.....	.....	.....	.....	.....	.....	.....	.....	2	2	3	3	.....	1
719	.....	.....	.....	.....	.....	.....	.....	.....	4	3	3	3	4	1
732	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5	4	4	1
737	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4	4	2	2
742	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3	3	4	1
744	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4	4	3	1
747	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3	3	3	0
754	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3	3	4	1
755	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	2	3	1

* *Rating code:* 5, Excellent; 4, Very Good; 3, Good Plus; 2, Good; 1, Fair.

each instance the type rating was given by an official inspector appointed by the Ayrshire Breeders' Association, who also indicated on a scorecard the main criticisms of each animal classified. The inspectors had no knowledge of any previous ratings on the animals until they had rated each animal and completed their work. The type rating standards of the official type classification program of the Ayrshire Breeders' Association—Excellent, Very Good, Good Plus, Good, and Fair—were used as the standard grades. In order to facilitate the analysis of the data, the official grades were coded, using 5 for Excellent, 4 for Very Good, 3 for Good Plus, 2 for Good, and 1 for Fair.

RESULTS

Variations in individual ratings. Eighty animals were officially rated three times or more. These ratings are shown in table 1, along with the dates classified, the inspector, and the range in the classification ratings for each cow.

A summary for the table shows that seven animals or 8.8 per cent were rated the same each time they were classified, 47 or 58.7 per cent had a range of one grade, 23 or 28.8 per cent had a range of two grades, and 3 or 3.7 per cent varied three grades. These results indicate that approximately one-third of the 80 head varied two or more grades when classified an average of five times over a period of 4.5 years by different inspectors. A further examination of the data was made to determine the major reasons for variations in type ratings.

Correlation between type ratings of the same cow when classified by the same inspector and by different inspectors. The relationships (repeatabilities) between ratings given a cow by the same inspector and between ratings given a cow by different inspectors were computed after the data first were analyzed by the analysis of variance (3). The mean squares for between cows and within cows were obtained and the components of variance for each mean square were determined. This analysis is shown in table 2, where the variation in type ratings of 53 cows classified by inspector R has been analyzed into differences between cows and within cows and the two components of variance, A and B, are calculated. The component of variance A is the variance caused by differences between ratings on the same cow, while B represents the additional variation that can be ascribed to differences between cows. The ratio $\frac{B}{A+B}$ represents the average correlation between the ratings on each cow classified by R and is 0.73. Inspector R classified the cows in May, 1942, October, 1942, and May, 1943.

Similarly, the correlations of repeatabilities of ratings of the same cow when classified by the same inspector were 0.82 and 0.62 for inspectors U

TABLE 2

Analysis of variance of type ratings of cows classified in the Reymann Memorial Herd by inspector E

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance components of mean square*	Individual components
Between cows ...	52	107.2	2.062	$A + 2.47\ B$	$B = 0.727$
Within cows	78	20.8	0.267	A	$A = 0.267$
Total	130	128.0	0.985	$A + 0.988\ B$	$A + B = 0.994$
				$\frac{B}{A+B} = 0.731$	

*Coefficients for the components of variance were computed by the method used by Dickerson (1).

and T, respectively, based on 42 and 9 cows. The classifications by inspector U were made in October, 1943, and January, 1944, while inspector T rated the cows in July, 1943, and May, 1946.

In table 3 the analysis of variance was used on the ratings of cows classified two or more times by different inspectors. In this analysis only the first rating placed on an animal by an inspector was used, and any later ratings given to the cow by the same inspector were omitted. The repeatability of ratings for these 101 cows was 0.55.

Tables 1, 2, and 3 indicate that there is a considerable amount of variation in the type ratings of individual Ayrshire cows. Some of the possible reasons for these variations, other than the difference in inspectors, were studied.

Effect of age on variation of type ratings. From observation it appeared that as cows advanced in age there was a tendency for the inspectors to raise their rating. In other words, it seemed that when an animal was classified at an early age, the inspector would rate the animal a grade lower if there was any doubt in his mind, because the animal always could be raised at a subsequent reclassification, but never officially lowered. Table 4 gives the average rating of all the cows classified at the ages of 2 to 11 years, inclusive.

TABLE 3

Analysis of variance of type ratings of cows classified in the Reymann Memorial Herd by different inspectors

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance components of mean square*	Individual components
Between cows ...	100	255.7	2.557	$A + 4.446\ B$	$B = 0.484$
Within cows	348	140.1	0.403	A	$A = 0.403$
Total	448	395.8	0.883	$A + 0.992$ B	$A + B = 0.887$
				$\frac{B}{A+B} = 0.546$	

* Coefficients for the components of variance were computed by the method used by Dickerson (1).

TABLE 4

Average type rating by age for all animals classified between the ages of 2 to 11 years, inclusive

Age classified	No. of animals	Av. rating
2	37	2.27
3	204	2.83
4	127	3.01
5	82	3.35
6	48	3.48
7	33	3.61
8	32	3.69
9	22	3.91
10	23	4.26
11	18	4.06

This table might lead one to believe that as the animals advanced in age the rating went up, but the factor of selection must be considered. The better type animals, as a rule, were kept in the herd longer, and those cows that were classified at 5 years and over were a select group of individuals. Averages as given in this table do not present an accurate picture of the effect of age on the variation of individual type ratings.

A more accurate picture is presented when the effect of age on type rating is studied within the same animals. In this study 65 females were included that were classified numerous times as 3-, 4-, and 5-year-olds. Thirty of the same females were classified several times as 4- and 5-year-olds, and 27 of them classified several times as 3-, 4-, and 5-year-olds. The results of this study are presented in table 5 and indicate that the change in type rating due to advancement in age is not great but is statistically significant between the ages of 4 and 5. More years of results will be needed to make the same determinations for older animals.

Effect of stage of lactation on variation of type ratings. During each calving interval a cow usually undergoes a constant change as far as her physical appearance is concerned. In the majority of cases dairy cows put on flesh during their dry period and when they come into production they are usually well covered with flesh and have a certain bloom that they lose during a heavy-producing lactation. The additional fleshing may serve to

TABLE 5

Average type rating by age within cows

Age classified	No. of animals	Av. rating
3	65	2.94
4	65	2.90
4	30	3.05
5	30	3.24
3	27	2.98
4	27	3.02
5	27	3.21

cover up certain faults; on the other hand, it may cause certain cows to appear to be lacking in dairy temperament. The appearance of the udder also changes constantly.

In an effort to determine some specific measure of the effect of stage of lactation on the variation of type ratings of the same cow, an analysis was made of the ratings of 63 animals classified 137 times between freshening and the fourth month of lactation, inclusive, 140 times between the fifth and the ninth month of lactation, inclusive, and 173 times between the tenth month of lactation and the next freshening period. The average of the ratings made between freshening and the fourth month, inclusive, was 3.27; between the fifth and the ninth month, 3.08; and from the tenth month of lactation to the next freshening period, 3.35. These differences are small, but are statistically significant when either the early or the last part of the lactation is compared with the middle segment. The data suggest that a little higher rating is obtained when animals are classified shortly before or after freshening, rather than in the middle part of their lactation.

As yet data are insufficient to make monthly comparisons of ratings throughout the lactation of a number of animals, which would be most desirable. Individual differences are great when cows are compared as to their changes in type conformation throughout a lactation period, and averages such as the above may cover up some of those wide individual differences. A photographic record kept on many of the cows that were classified at various times throughout their lactation is valuable and brings out very clearly how greatly some cows change in appearance, not only with stage of lactation but also with age.

DISCUSSION

The results of the project thus far seem to indicate quite definitely that the variations in type ratings of individual Ayrshire cows are considerable over a period of years. These variations are due to several causes, among which are age, stage of lactation, degree of fleshing of the animal, and the differences in inspectors. However, from an analysis of the data, along with the criticisms made of each cow by each inspector at each classification, most of the wide variations in type ratings of individual cows apparently could be attributed to inability of inspectors to attach equal significance to certain faults of a cow. This seemed particularly true when the animals were criticized for defects in udder, feet, and legs. For example, 22 of the 26 cows that had a range of two or more grades changed at least two official grades in two consecutive classifications (an interval of 3 to 6 months). For 19 of these animals the main faults recorded were crooked legs, bad feet, or udders defective in either shape or attachment. As a general rule, the inspectors agreed very closely as to their appraisal of faults other than those found in the feet, legs, and udders.

These results indicate that uniform cuts for the more important defects in feet, legs, and udder should be adopted. Perhaps this can best be accomplished through a series of pictures of cows that exemplify pictorially the type and seriousness of the defects involved. Then perhaps definite point cuts could be determined for the various types of faulty legs, feet, and udders, and these cuts applied by each inspector.

Obviously, the standardizing of the human factor is still the most perplexing problem in type classification work, and consequently, by deliberately planning to use the largest possible number of inspectors, variations in the results were invited. Type rating technique has been and is being improved and, no doubt, will continue to improve. However, the results of this experiment are quite similar to those found by Johnson and Lush (2) in their study of type in Holstein-Friesians,— namely, that two or more type ratings of an individual cow are a far more accurate guide to her true type conformation than is one official rating which can never be lowered irrespective of future conformation changes.

SUMMARY

Eighty Ayrshire cows at the West Virginia Agricultural Experiment Station were rated for type an average of five times over a period of 4.5 years by nine different inspectors. A summary of these results shows that 54 animals or 67.5 per cent had a range of one grade or less, while 26 or 32.5 per cent had a range of two or three grades.

The relationships (repeatabilities) between ratings given a cow by the same inspector at different times were calculated for three different inspectors and found to be 0.73, 0.82, and 0.62, while the repeatability of ratings given the same cows by different inspectors was calculated to be 0.55.

Type ratings were found to be somewhat higher on older cows and, in addition, were higher on cows classified during the first 3 months of a lactation or the last 2 months as compared with the middle segment of the lactation.

It is concluded that several type ratings on an individual cow form a far more accurate guide to her true type conformation than does one official rating which can never be lowered.

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THE UTILIZATION OF LACTOSE BY THE DAIRY CALF FED NORMAL OR MODIFIED MILK DIETS[1]

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Extensive studies with adult rats have shown that the addition of fat to skim milk diets markedly reduces the galactosuria observed when skim milk plus iron, copper, and manganese is fed as the sole diet (3, 8, 9). Little is known of the effect of fat on the utilization of lactose by the young calf or by other species. Results obtained on one calf and one pig (9) indicate that these animals perform in a manner similar to the rat. Both of these animals were found to utilize lactose completely when a whole milk diet was fed; however, after 2 weeks of skim milk feeding, considerable amounts of galactose were found in the urine. The galactose excretion apparently could be reduced to normal levels when 4 per cent corn oil was fed in addition to skim milk.

It was of importance, therefore, to extend these observations on the utilization of milk sugar by the young calf. The absorption and assimilation of lactose was studied when skim milk, whole milk, or modified whole milk diets were fed.

EXPERIMENTAL PROCEDURE

Ten male calves were used in these experiments, six Holsteins and four Jerseys. As these animals became available, they were fed either whole milk or skim milk diets. The group fed whole milk was composed of four calves, three Holsteins and one Jersey. Two of these animals received whole milk for the entire experimental period of 42 days, one calf was changed to a skim milk diet after 18 days, and the remaining calf was removed from the experiment after receiving whole milk for 12 days.

After the calves were taken from their dams, a daily milk allowance of 6 lb. for Jerseys and 8 lb. for Holsteins was pail-fed. The allowances were increased during the experiment according to the performance of the individual calf.

The group fed skim milk was composed of six calves, three Holsteins and three Jerseys. Two of these calves received a specially prepared low-fat colostrum for the first 4 days and skim milk thereafter; two suckled their dams for the first 24 hours; two received no colostrum and were fed skim milk from birth. Skim milk allowances were the same as for whole milk for the first 2 or 3 days, but were increased thereafter to compensate partially for the lower energy value of the skim milk.

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[1] Some of the data were taken from a thesis submitted by Jorge Rojas in partial fulfillment of the requirements for a Master of Science degree.

The skim milk diet was supplemented with 25,000 USP units of vitamin A per day by capsule as fish-liver oil and with approximately 1,000 units of vitamin D (viosterol). Since several investigators have shown that milk is low in iron, copper, manganese, and magnesium (1, 5, 6), the rations were supplemented with 7, 0.7, 0.6, and 43 mg., respectively, of these elements per pound of milk. Cases of scours or other digestive difficulties were treated successfully with 0.25 ounce of sulfaguanidine *per os* daily for 4 days.

All calves were kept in individual stalls with straw bedding, except during the days when urine collections were made. The urine was collected

FIG. 1. Apparatus used for making quantitative urine collections from young dairy calves. The apparatus is fastened firmly in order to avoid losses of urine when voided and can be adjusted according to the size of the animal. The animal is placed in a close-fitting elevated crate when urine collections are made, and the hose of the apparatus is passed through a hole in the bottom of the crate and fastened to a bottle placed under the crate.

for 24 hours at regular intervals. An apparatus and crate were designed to obtain quantitative urine collections. The apparatus used is shown in figure 1. The volume of urine voided was recorded and aliquots taken for sugar analyses.

Sugar determinations were made by the Shaffer-Hartmann method (10). The values for sugar were calculated by the use of the Munson-Walker tables (7) with the factor 1.22 to convert glucose values to galactose. It is recognized that urine contains reducing substances other than sugars which

TABLE 1

Effect of feeding whole milk on the urinary excretion of galactose

Periods	Days	Whole milk fed (lb. per day)	No. of calves	No. of urine collections	Galactose per 100 ml. urine (mg.)	Total galactose excreted (g. per day)	$\frac{\text{Galactose excreted}}{\text{Lactose ingested}} \times 100$
I	1–6	7.3	5	6	363.0	5.580	3.4*
II	7–12	7.8	4	8	131.4	3.142	1.8
III	13–18	9.0	3	4	118.0	3.112	1.5
IV	19–24	8.2	2	4	107.8	2.448	1.3
V	25–30	8.5	2	4	118.1	3.071	1.6
VI	31–36	9.0	2	4	81.5	2.218	1.1
VII	37–42	8.1	2	4	140.1	3.433	1.9

*Holstein calf no. 1139 excreted 12.274 g. galactose during the first collection.

may influence the values obtained. The results are expressed as galactose in all cases, although its presence was not confirmed by osazone tests. The actual values, therefore, cannot be interpreted unequivocally as galactose, but they serve as a useful measurement for comparing the effects of different dietary regimens, as has been widely used by other investigators. The accuracy of the method as applied to urine was checked, and recovery values for galactose added to urine were satisfactory, all within the range of ± 5 per cent of the theoretical.

RESULTS AND DISCUSSION

Previous reports on the utilization of lactose by rats fed skim milk diets were checked. Adult rats fed a skim milk diet for 2 weeks excreted an

TABLE 2

Effect of feeding skim milk on the urinary excretion of galactose

Periods	Days	Skim milk fed (lb. per day)	No. of calves	No. of urine collections	Galactose per 100 ml. urine (mg.)	Total galactose excreted (g. per day)	$\frac{\text{Galactose excreted}}{\text{Lactose ingested}} \times 100$
I	1–6	8.2	5	9	182.8	3.030	1.6
II	7–12	9.5	6	12	134.9	2.609	1.3
III	13–18	10.4	6	12	106.6	2.906	1.2
IV	19–24	11.6	7	14	121.2	4.149	1.6
V	25–30	11.7	6	10	120.8	3.319	1.3
VI	31–36	12.1	6	10	132.0	4.680	1.7
VII	37–42	12.3	5	8	115.4	4.751	1.7

average of 20 per cent of the ingested galactose. Substituting whole milk for skim milk in the diet reduced the galactose excretion to 2 per cent of the intake. These results confirm those of other investigators (3, 8, 9).

Summaries of the results for the groups of calves fed whole milk and skim milk diets are shown in tables 1 and 2. These data are subdivided into 6-day periods, which show that the amounts excreted did not increase as the experiments progressed. These results do not agree with those of Schantz *et al.* (9). In no case were values found for galactose excretion that approached the figure of 16 per cent of the intake reported by these investigators. The data show that only a small percentage of the galactose ingested was excreted in the urine, regardless of the dietary treatment used. Furthermore, no difference was observed in the values for galactose excretion between calves maintained on whole milk or skim milk diets for periods up to 42 days.

An inspection of the data for each individual animal failed to reveal any appreciable differences from the data shown as averages for all animals within each group. In some instances, an animal would show a sudden rise in urinary galactose (from 1 to 4 per cent of the intake) but immediately the amount excreted dropped to what can be considered normal. The variation that occurred in urinary galactose from day to day for individual

TABLE 3

Effects of feeding additional lactose to calves receiving skim milk diets

Days on experiment	Ration per calf per day	Total galactose excreted (g. per day)	$\frac{\text{Galactose excreted}}{\text{Lactose ingested}} \times 100$	Health remarks
	(Holstein calf no. 895)			
38–42	12 lb. skim milk	2.938*	1.1	Healthy appearance
44	12 lb. skim milk 320 g. lactose	24.832	4.2	Diarrhea
46	12 lb. skim milk 320 g. lactose	34.366	5.8	Diarrhea
48	12 lb. skim milk	9.706	3.6	Improved
51	12 lb. skim milk	9.034	3.3	Improved
	(Jersey calf no. A42)			
32–39	10 lb. skim milk	6.653†	2.9	Healthy appearance
40	10 lb. skim milk 250 g. lactose	19.458	4.1	Diarrhea
42	10 lb. skim milk 250 g. lactose	36.017	7.6	Diarrhea
44	10 lb. skim milk	18.693	8.2	Improved
46	10 lb. skim milk	5.849	2.6	Improved
48	10 lb. skim milk	11.792	5.2	Improved

* Average for 2 collections.
† Average for 3 collections.

animals and between different animals can be explained at least in part by variations in the volume of urine voided. This variation was appreciably less when the values were calculated for 100 ml. of urine. The percentage excreted was not dependent upon the amount of milk consumed. The calf maintained on whole milk for 18 days and subsequently on skim milk for 24 days failed to show any difference in the per cent of lactose excreted as galactose for the two periods (an average of 1.9 per cent when whole milk was fed and 1.7 per cent for the period when skim milk was fed).

Another calf 2 months of age that had been receiving hay and grain and subsequently was fed only skim milk for 4 weeks did not show any increase in sugar excretion when the latter feeding regimen was used.

Several fecal samples were collected to determine whether the lactose was being absorbed effectively, particularly by calves that were scouring. No appreciable quantities of reducing substances were detected in these samples; therefore, it appears that the calves were assimilating the ingested sugar efficiently.

In later phases of the work, after two of the calves had been fed skim milk for 42 days, the lactose content of their ration was doubled by the addition of lactose to the skim milk in order to determine the effect of increasing the percentage of lactose ingested on its utilization by the calf. The results obtained with these calves are shown in table 3. Addition of lactose to the milk decreased the percentage of sugar utilized by the animal. One of the calves excreted approximately 16 per cent of the ingested galactose. This figure approaches that reported by Schantz *et al.* (9) with skim milk feeding. Within a few hours after the lactose-enriched milk was fed, both calves were afflicted with diarrhea. This symptom has been observed in rats receiving high-lactose diets (2, 4). Additional work on the effect of feeding equivalent amounts of other carbohydrates would be valuable in order to determine whether the rise in sugar excretion occurs only with the ingestion of lactose.

Other observations on the performance of the calves fed whole milk or skim milk diets were of interest. Calves fed the skim milk diets made slower gains, undoubtedly due largely to the reduced caloric intake. Cases of scours occurred in calves fed skim milk as well as those fed whole milk; however, with the latter, a milder form was encountered. Most cases of scours responded satisfactorily to treatment with sulfaguanidine.

The data obtained in these experiments show that the young calf up to 3 months of age does not excrete appreciable quantities of galactose in the urine when receiving either whole milk or skim milk diets. Apparently the absence of fat in the diet does not interfere with the utilization of milk sugar by the young calf.

The work with the rat has been conducted with older animals, and no observations are available on the ability of suckling rats to utilize lactose when fed skim milk diets. Present results with calves suggest that the very young animal, being adapted to the milk diet, utilizes lactose efficiently. Further studies are needed to determine the difference, if any, between the very young and the older bovine in the utilization of lactose.

SUMMARY

1. The effect of feeding whole milk or modified milk diets to dairy calves on the amount of sugar excreted in the urine was determined. Equipment was designed for quantitative urine collection.

2. Calves fed whole milk or skim milk for periods up to 42 days utilized the dietary lactose very effectively. No difference was observed in the galactose excretions for the two groups. The amount excreted, measured as galactose, ranged from 1.1 to 3.4 per cent of the total lactose ingested.

3. When the lactose content of the milk was doubled, the result was an increase in urinary galactose equivalent to 8 per cent of the lactose ingested. The galactosuria was accompanied by diarrhea and unthriftiness of the animals.

The authors are indebted to Dr. F. W. Hill, Western Condensing Company, Appleton, Wisconsin, for supplying some of the lactose used in this study; to Mead, Johnson and Co. for supplying the vitamin D concentrate; and F. E. Booth Company for supplying some of the vitamin A concentrate used in this study. Mr. F. W. Taylor assisted with the care of the animals as part of a collaborative research program on the utilization of vitamin A and carotene by calves fed whole milk or modified milk diets.

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CONTENTS

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Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
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ABSTRACTS OF LITERATURE

BOOK REVIEWS

1. **Henrici's molds, yeasts, and actinomycetes.** 2nd Ed. C. E. SKINNER, C. W. EMMONS, AND H. M. TSUCHIYA. 409 pp. $5.00 John Wiley and Sons, Inc., New York, N. Y. 1947.

The authors have completely rewritten and considerably expanded the original edition, written in 1930. Their efforts have been directed toward the preparation of a book on yeasts, molds, and actinomycetes chiefly for students of bacteriology. In addition to expansion of previous chapters, new chapters have been included on variations in the lower fungi, infections caused by molds, pathogenic yeast-like fungi, and antibiotic substances. The revisions in classification are in some respects quite radical. Some of the suggestions undoubtedly will meet the approval of most mycologists, but others probably will meet considerable opposition. Examples of proposed changes in generic and species designation for some common dairy microorganisms are: *Geotrichum candidum* for the mold now known as *Oospora lactis* or *Oidium lactis, Cryptococcus sphaerica* for the yeast known as *Torula sphaerica, Scopulariopsis brevicaulis* for *Penicillium brevicaulis.*

The treatment of the various individual species of molds, yeasts, and actinomycetes is necessarily brief. In view of the present lack of any adequate source of information on many such organisms and their increasing importance in recent years, it is hoped that in future editions the authors will expand their descriptions and discussions in this field.

P.R.E.

2. **Practical emulsions.** 2nd Ed. H. BENNETT. 568 pp. $8.50. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1947.

After a general discussion of emulsions and emulsifying agents, specific technical applications are discussed by the author and by others, whose papers are presented in the form of a symposium on emulisfying agents and emulsions in industry. Lists of commercial surface-active agents, emulsifying agents, emulsions, and demulsifying and defoaming agents are given, with references to the original literature. A long section is concerned with formulas for emulsions for specific purposes. The sections concerned with food contain little of direct applicability to the dairy industry. F.E.N.

3. **The chemical composition of foods.** 2nd Ed. R. A. MCCANCE AND E. M. WIDDOWSON. 156 pp. $3.75. Chemical Publishing Co., Inc., New York, N. Y. 1947.

The data presented are the results of studies carried out by the authors

and their associates in Great Britain over a considerable period of time. The data are presented in two series of tables, one based upon 100-g. samples and one on 1-oz. samples, and recipes for the cooked foods analyzed are given in a separate section. Data on vitamin content are not given. Water, sugar, starch and dextrins, total nitrogen, protein, fat, available carbohydrate, 9 minerals, caloric value, and "acid-base balance" are given for each food. The various foods are arranged in groups, one of which consists of dairy products. F.E.N.

4. Food products. SAUL BLUMENTHAL. 986 pp. $12.00. Chemical Publishing Co., Inc., New York, N. Y. 1947.

This book is a compendium of formulas and procedures for the manufacture of a great variety of food products. A chapter is devoted to milk and milk products, and these materials are mentioned in a number of other places. Although the book contains a considerable amount of information, numerous errors are apparent, some of the material definitely is out of date and the treatment is spotty. Pages are devoted to a particular process, patent or minor product, whereas a topic as important as pasteurization of milk is dismissed with only cursory remarks and practically no specific information, and the same is true of a number of other important dairy products. A 5% DDT solution is recommended for insect control, but no information concerning formulation or specific methods of application is given. The sections of microbiology and the relationship of microorganisms to sanitation are very incomplete. Many of the references given in the brief bibliography found in the appendix have been superseded by more recent material. F.E.N.

BACTERIOLOGY

5. Observations on the use of a modified direct microscopic method for estimating bacterial quality of raw and pasteurized milk. G. W. WATROUS, JR., AND F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College. J. Milk and Food Technol., *10*, 5: 269–275. Sept.–Oct., 1947.

A modification of Newman Lampert formula no. 2 was recommended by using only 20% of the methylene blue dye and 20% of the glacial acetic acid as originally recommended. This stain gives a clear, light blue background, free of debris, with bacteria retaining the stain so tenaciously that identification easily was accomplished.

A ratio of one to one was attained with the modified stain and Levowitz's strip-counting technic on samples of raw milk when the plate and direct microscopic count were compared. There is no appreciable relationship

between this microscopic clump count and the plate count of organisms in fresh, laboratory-pasteurized milk. Non-viable organisms are capable of stain retention to a varying degree, depending on the microflora of the milk. Microscopic clump counts on fresh laboratory-pasteurized milk are lower than those prior to pasteurization, indicating that all the cells destroyed by pasteurization are capable of staining. A correlation exists between a high microscopic count in raw milk and the same milk after pasteurization. This relationship is not so striking where the bacterial content is relatively low in the raw and pasteurized milk. H.H.W.

6. **El B.C.G. en el Uruguay. Catorce años de experiencia sobre pasajes continuados en papa-bilis-glicerinada. (The B.C.G. in Uruguay. Fourteen years of experience with continuous passage on potato-bile-glycerine medium.)** A. TORTORELLA. Rev. med. vet., *4*, 43: 712–717. May, 1947.

A summary of a series of experiments that have been carried out on the B.C.G. bacillus for 14 yr. is given. The B.C.G. is not a changeable bacillus and therefore may be considered a definite strain. The successive passage of the organism in bile-potato-glycerine medium influenced neither the vitality of the bacillum nor its antigenic properties. The vaccine prepared from the B.C.G. strain, which had been grown and transferred in bile-potato-glycerine medium, showed a definite activity which was reflected in the following facts: (a) It grew abundantly when cultured; (b) it produced characteristic lesions in experimental animals, but these lesions were not progressive and the animals lived; (c) it produced positive tuberculin allergy in the guinea pigs used for the experiment, even 1 yr. after the inoculation; (d) it formed specific antibodies in the blood serum of experimental rabbits. R.E.M.

7. **La accion inhibitoria de la piocianina frente a las *Brucellas abortus, suis* y otros agentes. (The inhibitory action of pyocyanin against *Brucella abortus, suis* and other agents.)** N. PRADINES, Brazil. Rev. med. vet., *4*, 43: 718–726. May, 1947.

The organism *Pseudomonas pyocyanea* inhibited the growth of both *Brucella abortus* and *Brucella suis* in culture media. Pyocyanin, a water soluble blue pigment produced by the *Pseudomonas* organism, was found to have, by itself, the same action against the brucellas. This pigment had bacteriostatic and probably bactericidal, but not bacteriolytic, effects against those organisms. When tested *in vitro*, pyocyanin did not alter the phagocitic action of the white blood cells and retained its antibiotic action against the brucella organisms in the presence of blood serum. It also seemed to retain that property when tested *in vivo* in a chicken embryo.

Fluorescin, hemipyocyanin, and the antigen lipid-glucide, other products of *P. pyocyanea,* did not show any antibiotic action *in vitro* against the brucella species under consideration.

Preliminary experiments for a therapeutic value of pyocynanin *in vivo* against *B. abortus* and *B. suis* were carried out. The inoculation of pyocyanin did not cause any disturbances or lesions in small laboratory animals. The pigment easily was eliminated by the urine. Studies along this line still are being carried out and probably will be reported in the future. Pyocyanin also was found to have antibiotic action against *Pasteurella avicide.* R.E.M.

8. **Growth of *Staphylococcus aureus* in various pastry fillings.** W. H. CATHCART, W. J. GODKIN, AND G. BARNETT. The Great A & P Tea Co., New York, N. Y. Food Research, *12,* 2: 142–150. March–April, 1947.

Commercial dry-mixed puddings containing milk were found to support the growth of cultures of pathogenic *Staphylococcus aureus* when this organism was inoculated into them and the temperature made favorable. The organism was inhibited when water was substituted for the milk, but the quality of the pudding suffered. Vanilla, pumpkin, squash, and sweet potato pie fillings, cheese cake fillings, and whipping-cream mixes also were found to support the growth of this organism. By using citric acid to lower the pH of vanilla fillings to 3.43–3.65, the growth of the inoculant was checked but the filling acquired a sour taste. A better-tasting cheese cake filling was had by using lactic acid in place of citric; the staphylococcus was effectively inhibited at pH 4.42 to 4.67. F.J.D.

BUTTER

9. **Studies on the neutralization of cream for buttermaking. VII. Neutralization in practice in the butter-factory.** F. H. MCDOWALL, Dairy Research Inst. (N. Z), Palmerton North. New Zealand J. Sci. Technol., *28A,* 2: 132–138. Aug., 1946.

The amounts of sodium bicarbonate needed to neutralize creams of different acidities are presented in a chart and techniques employed during neutralization are described. W.C.F.

10. **Control of moisture content of butter during butter manufacture.** F. H. MCDOWALL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., *28A,* 1: 31–37. June, 1946.

The formula for calculation of the amount of water to be added to

partially-worked butter in a churn to bring the water content to a definite limit is as follows:

$$\text{Quantity to be added} = \frac{\text{desired } H_2O \text{ content (\%)} - \text{actual } H_2O \text{ content (\%)}}{100 - \text{actual } H_2O \text{ content (\%)}} \times \frac{\text{estimated load of butter in churn}}{1}$$

A chart calculated on the basis of this formula is presented; it is not applicable for calculation of salt to be added. Factors affecting the amount of water to be added are discussed. W.C.F.

11. **Land-cress taint in cream and butter. Parts I and II.** F. H. McDowall, I. D. Morton, and A. K. R. McDowell, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., *28A*, 5: 305–315. Feb. 1947.

Land-cress (*Coronopus didymus*) is an annual weed, of the family *Cruciferae*, which appears in young pastures and in bare patches in old pastures. When consumed by cows, it imparts an off flavor to milk and cream, butter, and cheese derived from it. Both land-cress and garden-cress contain a mustard-oil glucoside and both yield benzyl cyanide on direct steam distillation and benzyl isothiocyanate by the silver salt method. The distillate of land-cress also has a peculiar burnt odor. W.C.F.

12. **Land-cress taint in cream and butter. Part III. Relation of conditions of feeding land-cress to cows to incidence of land-cress taint in cream and butter.** F. H. McDowall, I. D. Morton, and J. J. O'Dea, Dairy Research Inst. (N. Z.), Palmerston North, and A. V. Allo, Dept. of Agr., Tauranga. New Zealand J. Sci. Technol., *28A*, 6: 370–384. April, 1947.

Consumption of as little as 2 oz. of cress by cows caused taint in the cream from milk drawn within 30 min. from the time of eating. When cows ate the cress at noon, the taint was strong in the cream of milk drawn 9 hr. later and was present in milk drawn the following morning. Thus, removal of cows from pastures containing the cress did not prevent the taint in the following milkings but did cause a decrease in the intensity of the defect. Land-cress hay caused a weak taint in the cream. Measures are described for the prevention of the growth of the cress in pastures. If the weed is present, only dry stock should be pastured there. W.C.F.

13. **The washing of butter and its effect on curd content and quality.** E. G. Pont, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), *19*, 4: 432–437. Nov., 1946.

See Abs. 279, J. Dairy Sci., *30*, 9: A122.

CHEESE

14. A practical system for determining the premium value for high fat Cheddar cheese. A. B. Erekson, Plymouth, Wis. Natl. Butter Cheese J., *38*, 9: 44. Sept., 1947.

This discussion is a development of a previous article. (See Abs. 311, J. Dairy Sci., *29:* A144. 1946.) It shows that when a working standard of 52.5% fat in the dry matter of the cheese is adopted, higher amounts of fat command a premium and lower amounts a deduction from the market price of the cheese. The value of the fat is obtained by: (a) multiplying the price per pound of 92 score butter by 1.15 to get the approximate value of fat in cream; and (b) deducting the value of 1 lb. of cheese solids. This latter value is calculated by dividing the price of cheese by 0.61, the minimum amount of dry matter permitted by law in a lb. of Cheddar cheese. A table showing the value of fat for numerous butter and cheese price combinations is given. W.V.P.

15. Factors influencing the texture of Cheddar cheese. G. H. Wilster, Oregon State College, Corvallis. Natl. Butter Cheese J., *38*, 10: 48. Oct., 1947.

The texture of cheese is improved by using high-grade milk, even though the milk is pasteurized before making the cheese. Other essentials for cheese of the best texture are: clean, active starter, efficient pasteurization, clean equipment, acidity control, moisture control, and control of temperatures and pressures during cheddaring and pressing of the curd. These means for improving and controlling texture are suggested for treatments of specific defects, including gassy, greasy, open and crumbly texture, and corky and mealy-pasty body and texture. W.V.P.

16. Pasteurização a jato direto. (Direct steam pasteurization.) J. A. Ribeiro. Boletim do leite, *1* (4ª Epoca), 1: 9–14. July, 1947.

A direct steam pasteurization process to be employed for milk that will be used in the manufacture of cheese is described in detail and a few drawings are given to illustrate it. The advantages credited to this process are: (a) It is a cheap form of pasteurization. (b) It is easy to use. (c) It uses a limited space in the cheese plant. (d) It takes advantage of the vacuum created by the steam injection to raise the milk to higher levels, saving time and human effort. Studies have not been made of the comparative bacteriological efficiency of the procedure. R.E.M.

17. Fermentos para queijos. O fermento lático é a alma do queijo. O emprego de fermento perfeito é a base da obtenção de queijos

ótimos. (Ferments for cheese. The lactic ferment is the foundation of the cheese. The use of the perfect ferment is the base in the obtention of optimum cheeses.) J. A. RIBEIRO. Boletim do leite, *1*, 9: 1–4, 18. June, 1947.

A brief and general discussion of the different organisms that influence the ripening of the different types of cheeses is given. A recognized method for the day-to-day preparation of the starter, for the manufacture of semi-hard cheeses from pasteurized milk, is presented in detail. The aseptic handling of the cultures throughout the preparation of the starter is emphasized and the beneficial effects of the use of starter in the manufacture of semi-hard cheeses from pasteurized milk are enumerated. R.E.M.

18. **Temperature and humidity control in cheese-curing rooms.** T. R. VERNON, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., *28A*, 6: 361–369. April, 1947.

Cheese was of better quality after storage in an insulated room controlled automatically at 55° F. and a humidity of 85% than after storage in an uninsulated room or an insulated room without such controls. Some trouble with mold growth was encountered in the controlled room, but weight losses were less. W.C.F.

19. **Control measures against the cheese-mites, *Tyrolichus casei* Ouds. and *Tyrophagus longior* Gerv.** J. MUGGERIDGE, Plant Research Bureau, Nelson, AND R. M. DOLBY, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., *28A*, 1: 1–30. June, 1946.

Wax protected cheese somewhat against mites, but they were able to bore through the wax layer and enter the cheese. Dusts were ineffective against attacks by mites, and the high humidity necessary in cheese warehouses made the use of dusts difficult. Three fumigants, ammonia, methyl bromide, and dichloroethyl ether, were found effective, with the latter considered the most suitable. It is effective in low concentrations, is easily handled and applied, and is cheap. The chemical may be used in the vapor state as a fumigant, but the authors recommend that it be applied as a liquid to the shelves in the curing room at the rate of 1 lb. per 1,000 cu. ft. of room space or per 100 sq. ft. of shelving. Another method is to place the cheeses on boards treated with the chemical. Methyl bromide is expensive and is not as persistent as dichloroethyl ether. Ammonia is too readily absorbed by surrounding materials. W.C.F.

20. **Cheese and its relation to disease.** CURRENT COMMENT. J. Am. Med. Assoc., *135*, 11: 718. Nov. 15, 1947.

This is a detailed review of the paper of the same title by F. W. Fabian, Am. J. Pub. Health, *37*: 987. Aug., 1947.

CHEMISTRY

21. The thiamin, riboflavin and niacin content of some New Zealand milks. F. H. McDowall, Dairy Res. Inst. (N. Z.), N. O. Bathurst, Plant Chem. Laboratory, and I. L. Campbell, Dairy Research Inst., Palmerston North. New Zealand J. Sci. Technol., *28A*, 5: 316–328. Feb., 1947.

Thiamin was estimated by the fluorimetric method and niacin and riboflavin were measured by microbiological methods. The thiamin and riboflavin contents of Jersey milks regularly were higher than those of Friesian milks, but the niacin content was the same for both breeds. The feeding of concentrates to cows on pasture caused a rise in the thiamin content of milk from Jersey cows but not from Friesian cows. W.C.F.

22. The polarographic estimation of ascorbic acid in milk. Dawn R. Perrin, Dairy Laboratory, Wallaceville, and D. D. Perrin, Animal Research Sta., Wallaceville, New Zealand Dept. of Agr. New Zealand J. Sci. Technol., *28A*, 4: 266–272. Dec., 1946.

The technique was employed for the estimation of ascorbic acid in milk but it also could be used to measure dehydro-ascorbic acid. W.C.F.

23. The estimation of copper in cream. G. C. DeAth, F. Ruth Lightfoot, and G. M. Moir, Dairy Div. Laboratory, Dept. of Agr., Wallaceville. New Zealand J. Sci. Technol., *28A*, 4: 273–284. Dec., 1946.

This is a modification of the filtration method used with butter. Improvements in the wet-ashing method also are described. W.C.F.

24. Determination of iron in foods and food products. W. D. Pohle, J. H. Cook, and V. C. Mehlenbacher. Research Laboratories, Swift and Co., Chicago, Ill. Food Research, *12*, 3: 229–238. May–June, 1947.

A simple, accurate colorimetric method is described for the determination of iron in food products employing dry ashing and color development with 1, 10-phenanthroline. Phosphates and copper in normal quantities did not interfere. The method was applied to milk and dried milk satisfactorily. F.J.D.

25. Note on the effect of phosphatide on the ferric thiocyanate method of estimating peroxide in fats. G. L. Hills and R. Wilkinson, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), *19*, 4: 430–431. Nov., 1946.

See Abs. 287, J. Dairy Sci., *30*, 9: A124.

26. The mechanism of the oxidant effects of commercial salt and water in butterfat. G. L. Hills and J. Conochie, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), *19*, 4: 414–429. Nov., 1946.

See Abs. 286, J. Dairy Sci., *30*, A124.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

27. A rapid method for the estimation of whey proteins as an indication of baking quality of non-fat dry milk solids. H. A. Harland and U. S. Ashworth, Agr. Expt. Sta., Pullman, Wash. Food Research, *12*, 3: 247–251. May–June, 1947.

A method for estimating the baking quality of milk proteins in the form of dry milk, concentrated milk, and fluid milk is presented. The presence of a low concentration of whey proteins (indicating relatively high heat treatment) is shown to correlate with good baking quality. The whey proteins are determined, after the casein of the sample has been salted out, by acidifying the diluted filtrate and measuring the turbidity by means of a colorimeter. F.J.D.

28. The utilization of buttermilk in New Zealand. F. H. McDowall, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., *28A*, 2: 97–132. Aug., 1946.

In 1940–1944, buttermilk from New Zealand creameries was used almost exclusively for animal feeding. Variations in the composition of buttermilk are discussed and other uses for the product are described. W.C.F.

29. Directions for making kefir fermented milk. L. A. Burkey, Bureau of Dairy Ind., U. S. Dept. Agr. BDIM-Inf.-58. 4 pp. Nov., 1947.

Concise directions for handling kefir grains, for preparation of the milk and for handling of the product are given for both small and large scale manufacture. F.E.N.

FEEDS AND FEEDING

30. The effect of the ingestion of high levels of riboflavin on the amount in the milk and urine. P. B. Pearson and B. S. Schweigert, Agr. and Mech. College of Texas, College Station. J. Nutrition, *34*, 4: 443–453. Oct. 10, 1947.

Riboflavin values of normal goat and sheep milk as measured microbiologically and fluorimetrically agreed closely. After feeding 2 g. of synthetic riboflavin, the microbiological procedure showed an increase in the

riboflavin content of the milk of about 26%. However, the fluorimetric procedure gave values for the milk of about 5 times greater than the microbiological technique. Growth responses of rats fed the milk agreed with the microbiologically determined values rather than the fluorimetric values.

The microbiological and fluorimetric values for riboflavin content of urine of rats fed large amounts (10 or 20 mg.) of riboflavin agreed closely. Both techniques gave similar values for urine from humans fed 20 mg. of riboflavin per day. Goats and sheep fed large amounts of riboflavin evidently metabolize some of the vitamin into a fluorescent compound which is biologically inactive. R.K.W.

31. Vitamin A intake in cattle in relation to hepatic stores and blood levels. P. H. FREY, R. JENSON, AND W. E. CONNELL. Colorado Agr. Expt. Sta., Ft. Collins. J. Nutrition, *34*, 4: 421–430. Oct. 10, 1947.

Hereford steers of about 8 months of age were fed a carotene-free basal diet plus vitamin A supplements of 0, 25, 200, and 500 I.U. daily per lb. of body weight. Serum levels of vitamin A and carotene were determined at 0, 27, 83, 159, and 277 days and hepatic values at 0, 166, and 280 days. Animals receiving vitamin A supplements all grew at about the same rate. Dietary vitamin A did not exert a sparing action on hepatic stores of carotene. Vitamin A stores of the liver increased in practically a linear relationship with increased intake. Serum levels of vitamin A increased rapidly up to a daily intake of 100 I.U. per lb. of body weight, when the serum contained about 50 mμ per 100 ml. at 280 days. R.K.W.

FOOD VALUE OF DAIRY PRODUCTS

32. Some studies on the nutritive value of butter fatty acids. E. HEFTMANN, Univ. of Rochester. J. Nutrition, *34*, 4: 455–467. Oct. 10, 1947.

No significant difference in gain of weights or efficiency of food utilization was found between two groups of young rats (5 males and 5 females in each group) when one group received butter fatty acids and the other group received the non-volatile fatty acids of butter for a feeding period of 5 weeks. Gains of weight and efficiency of food utilization were decreased only in the male rats fed hydrogenated fatty acids when one group received the butter fatty acids and the other group received partially hydrogenated fatty acids; intakes of vitamin A and D were low. In still another trial, the amounts and concentrations of vitamin A stored in the livers of rats fed preformed vitamin A were not significantly affected by complete hydrogenation of the butter fatty acids. R.K.W.

HERD MANAGEMENT

33. The influence of certain milking-machine adjustments on the rate of machine-milking. W. G. WHITTLESTON AND S. A. VERRALL, Animal Research Sta., Dept. of Agr., Ruakura. New Zealand J. Sci. Technol., *28A*, 6: 406–416. April, 1947.

Variations in pulsator speeds (21, 42, and 84 pulsations per min.) and in vacuums (10, 14.75, and 19 in.) did not cause a significant difference in the milking rate. W.C.F.

34. Milking apparatus. L. F. BENDER AND J. A. SCHMITT. (Assigned to Universal Milking Machine Co.) U. S. Patent 2,429,983, Nov. 4, 1947 (14 claims). Official Gaz. U. S. Pat. Office, *604*, 1: 85. 1947.

A teat cup claw is described, the bottom of which easily may be opened for inspection. Suitable connections are provided for collecting the milk from the 4 teat cups and delivering it through a hose to a milk-collecting device. Nipples also are provided for the air which operates the teat cups alternately. R.W.

35. Comparative rates of production of different breeds of dairy cattle. E. G. MISNER. Holstein-Friesian World, *44*, 21: 2696–2697. Nov. 1, 1947.

A summary of 1,616 sires proved in D.H.I.A. work in 1945 shows that their daughters, arranged by breeds, averaged 178 lb. less milk, 2 lb. less fat, $5 less energy value of product, and 0.05 more in fat percentage than their dams. The author interprets these figures to mean that: (a) the sires used are not so good as they should be to maintain the rate of production of our dairy herds or (b) the data are not fair to the bulls. A factor for correctly converting the total fat production to mature total fat production may be too low for converting milk production on young cows and may not give any consideration for decreasing test due to age.
A.R.P.

36. The persistence of DDT on cattle. R. H. HACKMAN, Div. of Ind. Chem. J. Council Sci. Ind. Research (Australia), *20*, 1: 56–65. Feb., 1947.

Licking, either by the animal concerned or by another animal, is the most important factor causing the removal of DDT from cattle sprayed with that chemical. The amount of DDT ingested in this way is insufficient to produce toxic symptoms. W.C.F.

37. Stock watering tank. C. FINE. U. S. Patent 2,430,165, Nov. 4, 1947 (3 claims). Official Gaz. U. S. Pat. Office, *604*, 1: 131. 1947.

The chief feature of this tank is a tubular chamber spaced above the

bottom, which acts as a fire box for providing heat. The smoke is discharged through a chimney extending upward from the chamber. R.W.

38. Electrical system for cattle stalls. J. J. HANTZ. U. S. Patent 2,428,875, Oct. 14, 1947 (6 claims). Official Gaz. U. S. Pat. Office, *603*, 2: 264. 1947.

To improve the sanitary condition of dairy cattle stalls an electrical device is used to cause the cows consistently to deposit the droppings in the gutter back of the stall. The device consists of a horizontal bar, charged with a small electric current, adjustably suspended a few inches over the cow's back. If the cow is not standing in the proper position, she receives a small shock at the time the back is arched prior to evacuation, thereby causing the cow to step backward into position to deposit the droppings into the gutter. R.W.

MILK

39. The significance of the coliform test in pasteurized milk. P. D. DELAY, California Agr. Expt. Sta., Berkeley. J. Milk and Food Technol., *10*, 5: 297–299. Sept.–Oct., 1947.

The coliform test is an aid in detecting faulty post-pasteurization handling. The results of this study indicate that well-equipped plants can, without exceeding the limits of practicability, handle milk which will be less than 1% positive for the coliform test. The present maximum level of less than 5 to 10% is maintained. The ultimate goal is to attain the 1% limit of coliform organisms in pasteurized milk by a gradual process, depending upon existing conditions in a given locality. H.H.W.

40. Agregado de sustancias quimicas a la leche para su conservacion. (Addition of chemical substances to milk for its preservation.) L. J. MURGUIA. Rev. med. vet., *4*, 43: 702–711. May, 1947.

Use of hydrogen peroxide, formol, sodium bicarbonate, oxygen, ozone, and trichloronitromethane or "microlysine", chemical substances that have been used to prevent the milk from curdling, was reviewed as a result of the recent use of "microlysine" as a milk preservative in France. Use of any one of the substances under consideration was discouraged and condemned as a backward step in milk sanitation. R.E.M.

PHYSIOLOGY

41. The characteristics of the milk-ejection curve of normal dairy cows under standard milking conditions. W. G. WHITTLESTON, Ruakura

Animal Research Sta., Dept. of Agr. New Zealand J. Sci. Technol., *28A*, 3: 188–205. Oct., 1946.

To ascertain quantitatively what happens when cows are milked mechanically without hand stripping, the milk-ejection curves of a dozen cows were recorded throughout one season by means of a milk-flow recording apparatus. The average rate of milk flow tended to decline toward the end of the season, so that the time of milking did not decrease appreciably. The machine strippings did not increase significantly with declining yield, but the percentage of milk yielded as strippings increased. The starting time tended to increase and become erratic toward the end of lactation. No factor in the strict milking-machine procedure appeared to be harmful to the health of the udder. W.C.F.

42. Contribucion al estudio del Calcio y Fosforo inorganico en la sangre de la vaca ledhera. (Contribution to the study of inorganic calcium and phosphorus of the blood of the milk cow.) F. A. ROJAS. Agricultura tecnica, *7*, 1: 20–25. June, 1947.

The amounts of Ca and P in the blood serum of 101 lactating cows, which represented different breeds and different levels of production, were determined. The blood samples were taken before the animals were allowed to eat in the morning. The Ca was found to be present in the range of 8 to 10.8 mg. per 100 cc. of blood serum, with 9.37 mg. as the average. P was present in the range of 2.70 to 7.50 mg. per 100 cc. of blood serum, with 4.77 mg. as the average. The Ca-P ratio fluctuated between 1.70 to 1 and 2.65 to 1, the average being 2.15 to 1. The Ca and P contents found were lower, in general, than those obtained by Haag and Jones in similar experiments. R.E.M.

SANITATION AND CLEANSING

43. A study of the germicidal efficiency of can washing compounds. M. J. FOTER AND R. D. FINLEY, Research Laboratory, Pet Milk Co., Greenville, Ill. J. Milk and Food Technol., *10*, 5: 257–262. Sept.–Oct., 1947.

The germicidal efficiency of 6 alkaline and 1 acid can-washing compounds was studied in 120 to 130 milk cans. The alkalinity levels were studied within different ranges. The germicidal efficiency was very slight or non-existent at an alkalinity range of 0.05 to 0.10% as Na_2O. When the alkalinity was increased to 0.15 to 0.20% as Na_2O, the germicidal effect was increased markedly with 2 of the cleaners.

When the acid can-washing compound was used, the acidity of the wash solution was neutralized by the calcium and magnesium salts of the hard water. This condition produced an alkaline reaction in the milk cans.

The germicidal efficiency of the acid cleaner was increased if the cleaner was maintained at a point at which the cans were acid.

The authors suggest that careful consideration should be given to the cleansing and germicidal efficiency of washing compounds in addition to rinsability, wetting, emulsification, water conditioning, etc. Germicides are not routinely used in mechanical can washers. Too much dependency should not be placed on the temperature of the rinse water, sterile rinse, temperature of the steam, and hot air for drying. H.H.W.

44. A study of the corrosion of tin plate by can washing compounds. R. D. FINLEY AND M. J. FOTER, Research Laboratory, Pet Milk Co., Greenville, Ill. J. Milk and Food Technol., *10*, 5: 263–268. Sept.–Oct., 1947.

The corrosive action on tin plate was studied by testing 8 commercial alkaline cleaners, 2 commercial acid cleaners, and 4 basic alkalis. All of the commercial alkaline cleaners and basic alkalis readily attacked tin, while the two acid cleaners had very little effect on the tin during the time of exposure. The acid cleaners in some cases showed spots of corrosion, with formation of pits. The corrosive action of sodium hydroxide adjusted to a wide concentration range had very little effect on the tin, except when a very low concentration was used. The oxygen content of the alkali solution probably plays a major rôle in tin corrosion.

The can washing alkalinity range generally used is 0.05 to 0.20% Na_2O, which is equivalent to 0.005 to 0.27% NaOH. This strength fell within the sodium hydroxide concentration range where maximum corrosion occurred. Results seem to indicate that a corrosive inhibitor, such as sodium sulfite, should be added to alkaline can washing compounds. An inhibitor would minimize alkaline corrosion of cans and also increase the germicidal action of alkalies in cleaning compounds. H.H.W.

45. The toxicity to houseflies of paints containing DDT. D. GILMOUR, Div. of Econ. Entomol. J. Council Sci. Ind. Research (Australia), *19*, 3: 225–232. Aug., 1946.

DDT was added to a glossy enamel, a flat oil paint, an emulsion-type paint, a water paint, and an ordinary oil paint. The most effective mixtures were a glossy enamel containing 20% DDT and a flat oil paint with 3–5% DDT. The effectiveness of the oil paints depended on the degree to which the DDT had crystallized in the film. W.C.F.

MISCELLANEOUS

46. Pasteurizing apparatus. E. K. KINTNER. (Assigned to Arco Welding and Machine Works.) U. S. Patent 2,428,880, Oct. 14, 1947 (3 claims). Official Gaz. U. S. Pat. Office, *603*, 2: 266. 1947.

A pasteurizer of the plate heat-exchanger type is described. A rubber gasket vulcanized to the perimeter of each plate prevents leakage. The equipment is characterized by a centrally located baffle which causes the liquid to flow up one side and down the other between any two plates, the direction being reversed between the next two plates for maximum efficiency of heating or cooling. R.W.

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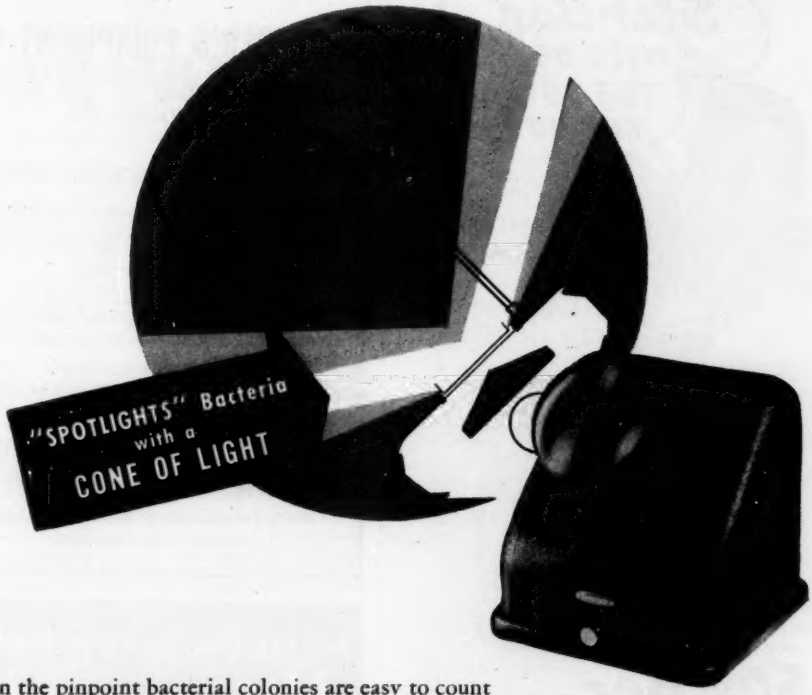

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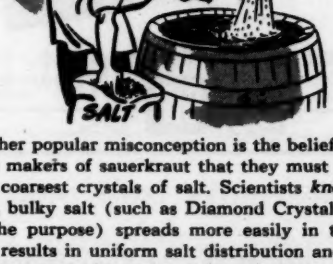

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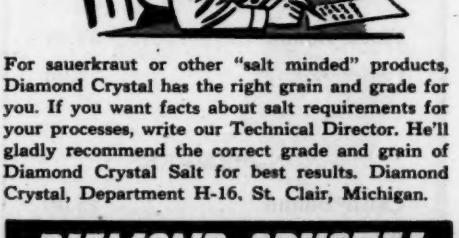

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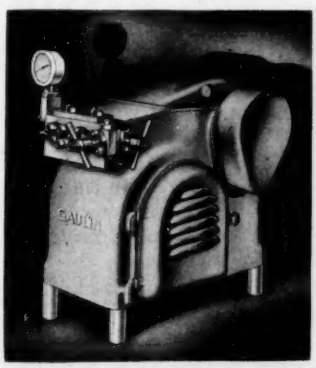

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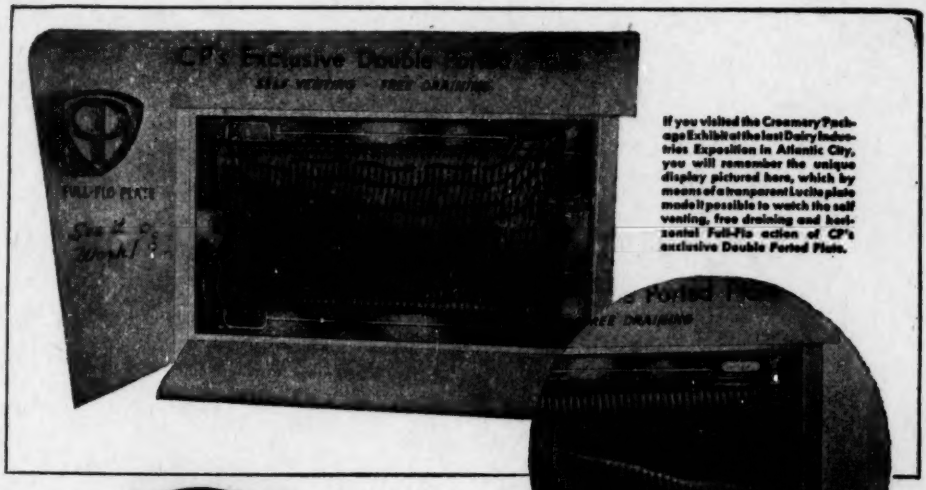

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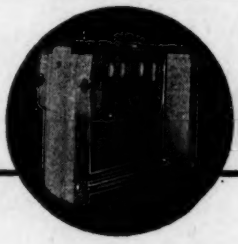

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